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JP-8 Final Risk Assessment

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The JP-8 Final Risk Assessment is a collection of several studies assessing the health and performance effects from acute exposure to Jet Propellant type 8 (JP-8) jet fuel. Exposed subjects were active duty Air Force personnel who worked with or were routinely exposed to JP-8 in the performance of their duties. Non-exposed subjects were active duty Air Force personnel who did not have routine contact with JP-8 during the performance of their duties. Assessments included JP-8 exposure in the immediate personal environment, JP-8 body burden, neurologic effects, hormonal effects, immunological effects, liver/renal function, cytotoxic/genotoxic effects, glutathione-S-transferase activity, and self-reported health status.

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EXECUTIVE SUMMARY

The following report represents the final report of preliminary results of the protocol to assess the health and performance effects of acute exposure to Jet Fuel number 8. Texas Tech University, Institute of Environmental and Human Health, in conjunction with the United States Air Force, hosted this protocol with funding from Strategic Environmental Research and Development Program. Additional collaborators include the University of Cincinnati, and the Oregon Health Sciences University, the University of Texas, the University of North Carolina, Johns Hopkins University, the US Navy Toxicology Laboratory at Wright-Patterson AFB, OH, the NIOSH, and EPA/NERL herein referred to as the JP-8 Research Team.

Jet Propellant type 8 (JP8) jet fuel is the recognized battlefield fuel for all military operations for the United States, well beyond the year 2025, and represents the single largest source of chemical exposure to Department of Defense (DOD) personnel. Currently, DOD and its NATO partners use approximately 5 billion gallons of JP8 annually. The commercial equivalent, Jet-A, is the primary jet fuel used by aircraft in the US. Worldwide use of kerosene-based jet fuel is over 58 billion gallons per year

The study was conducted at multiple Air Force installations. Dyess AFB, TX, served as the beta test site for participant selection, specimen collection, and exposure assessment. The lessons learned from the Dyess AFB beta test allowed the JP8 Research Team to improve data collection processes and study logistics, thus reducing the operational study impact at other Air Force bases involved in the study. Data was collected at the following sites: Davis Monthan AFB, AZ, Seymour Johnson AFB, NC, Langley AFB, VA, Pope AFB, NC, Little Rock AFB, AR, and Hurlbert Field, FL. Specimens and data collected from these locations were analyzed at established laboratory facilities operated by the universities and government agencies involved in the study

Two groups of airmen were enrolled. Those designated as **JP8 exposed** consisted of active duty Air Force personnel who routinely worked with or are exposed to JP8 in the performance of their duties. Most exposed volunteers worked in Aircraft Fuel Cell Maintenance shops. These workers routinely performed maintenance activities requiring entry into aircraft fuel tanks. Other exposed volunteers worked in either the Fuels Specialty or Fuels Transportation shops. In order to qualify for the study, exposed volunteers were required to have least 9 months of persistent exposure to jet fuel (such as fuel tank entry at least one hour twice weekly).

Unexposed volunteers were intended to represent the population of active duty Air Force personnel assigned to the Air Force installation where the study was being conducted. They consisted of active duty personnel assigned to the same Air Force installation as the JP8 exposed volunteers, but who do not have routine contact with JP8 or other fuels during the performance of their duty. A wide variety of job classifications were represented in the unexposed group. Since nearly all JP8 exposed volunteers were enlisted personnel, attention was paid to ensure, for the most part, that Air Force officers were not selected to participate. In rare cases, officers were included where the researchers felt their inclusion would not bias the analysis.

Broadly, JP8 exposure was measured both externally in the environment immediately surrounding enrolled workers and internally through the use of several body burden measures. The impact of exposure was evaluated using a series of neurological, hormonal and immunological measures. Cytotoxic and genotoxic effects of JP8 exposure were also evaluated. The activity of Glutathione-S-Transferase (G-S-T), a gene-regulated enzyme associated with increased susceptibility to multiple oxidative stressors including jet fuel and linked to adverse health outcomes, was also measured. Self-reported health problems, health care visit frequency, and early indicators of liver and kidney damage were investigated as part of the study.

The characterization of JP8 health risks, conducted by Texas Tech University,and the identification of uncertainties accounted for exposure measures of JP8 and measures of effect. The preliminary risk characterization attempted to determine the association between the various measures of effect used in this study and assesses the overall impact, by JP8 dose, on workers exposed to the fuel. However, to thoroughly utilized the collected data for risk assessment, additional financial resource is required to keep the JP-8 Research Team in place to continue exploring the questions raised at the International Conference on JP-8 Jet Fuel, August 7-10, 2001, San Antonio Texas.

INTRODUCTION

Risk Assessment of Acute Exposure to Jet Fuel

Jet Propellant type 8 (JP8) jet fuel is the recognized battlefield fuel for all military operations for the United States, well beyond the year 2025, and represents the single largest source of chemical exposure to Department of Defense (DOD) personnel. Currently, DOD and its NATO partners use approximately 5 billion gallons of JP8 annually. The commercial equivalent, Jet-A, is the primary jet fuel used by aircraft in the US. Worldwide use of kerosene-based jet fuel is over 58 billion gallons per year.

Over the past 20 years, JP8 largely replaced JP4 as the primary aircraft fuel for U.S. military aircraft. JP4, which is chemically similar to gasoline, is highly volatile. Explosive fires in both occupational and operational settings were experienced in military aircraft powered by JP4. JP8, although chemically similar to kerosene, is much less volatile. It is a much safer fuel to handle and less likely to propagate an explosion during instances when military aircraft fuel tanks suffer artillery or small arms damage during operational situations.

As JP8 was phased into the military inventory, exposed personnel began voicing concerns about the potential health effects of exposure. Aircraft groundcrew members reported objectionable odors, skin irritation, dizziness and the persistent taste of jet fuel long after exposure. These concerns prompted the Air Force Surgeon General to task the Air Force Institute for Environment, Safety and Occupational Health Risk Analysis (AFIERA) and the Air Force Research Laboratory (AFRL) to address personal exposure and toxicological hazards from JP8.

A reference report by the Center for Disease Control's (CDC) Agency for Toxic Substances and Disease Registry (ATSDR), "Toxicology Profile for Jet Fuels (JP-5 and JP8)," in 1997 indicated that the toxicities of jet fuel and their mechanisms are not well-defined. According to ATSDR, data gaps exist on dose-response, reproductive system, developmental effects, immune system, neurological system, biomarkers of exposure and effect, rates of absorption, distribution and excretion of, and toxicokinetics in current research of human health effects from jet fuel exposure. Recently, JP8 jet fuel was selected as a priority hazardous chemical requiring establishment of an acute exposure limit by the Environmental Protection Agency's (EPA) National Advisory Committee for Acute Exposure Guidelines for Hazardous Substances (NAC-AEGL), a sub-committee of the Office of Pollution Prevention and Toxics, US EPA. The NAC-AEGL further identified data gaps in the toxicology profile of jet fuel as submitted by the ATSDR. Recommendations from the NAC-AEGL include measuring total body burden, identifying biomarkers of exposure, conducting an epidemiology study of worst-case exposed populations, conducting neurological assessment, establishing reference dose (RfD) and risk assessment of exposure from JP8. In addition, a 1996 report by the National Research Council's Committee on Toxicology (COT) identified data gaps in occupational exposure assessments, breath analysis, quantitative neurological effects and hepatotoxicity.

The COT report recommended the following:

a) Obtain information on exposures during operational procedures, including exposures to respirable aerosols of unburned fuels.

b) Conduct studies on the possible effects of high-level acute and low-level chronic exposures to military fuel vapors on CNS, including the effects on performance of military personnel.

c) Conduct further research on the effect of military fuel vapors on hepatotoxicity in experimental animals.

Based on the Air Force Surgeon General's tasking and ATSDR, COT and NAC-AEGL recommendations, AFIERA initiated a program to evaluate all environmental, safety and occupational health aspects of jet fuel and began collaboration with the National Institute for Occupational Safety and Health (NIOSH), the Environmental Protection Agency, National Exposure Research Laboratory (EPA-NERL), the National Institute for Environmental Health Sciences (NIEHS), and selected academic institutions to resolve open issues regarding JP8. The USAF JP8 Environmental, Safety and Occupational Health Integrated Process Team (IPT), formed in 1996 and in coordination with the Air Force Office of Scientific Research (AFOSR), conducted and funded animal toxicology studies on aerosol exposure, dermal flux and adsorption, biomarkers and neurological assessments. The IPT has also conducted and funded occupational exposure studies to include ambient vapor and aerosol exposure assessment, breath sampling, and heat stress assessment.

Based on exposure data from previous AFIERA studies, fuel tank repair operations of single-point-entry fuel bladders containing fire suppressant foam were determined as the worst-case exposure situations. The highest exposure results were measured in operations performed in the C-130 Hercules transport aircraft's auxiliary fuel tanks.

The studies conducted by AFIERA and other investigators, including toxicology studies supported by the AFOSR, validated the need for research on JP8 impact on workers in occupational settings. In particular, studies of the acute effects of exposure were considered most important. Based on these assessments, this study, entitled **Risk Assessment of Acute Exposure to Jet Fuel**, was developed and initiated.

Prior to this study, no occupational exposure cohort studies had been conducted to assess the effects from acute exposure to JP8 jet fuel. Further, no acute exposure or risk assessment studies had attempted to link quantitative neurological measurements to ambient exposure, biomarkers, and total body burden. This study breaks new ground by correlating ambient exposure with human body burden and neurological performance measures. The results of this study are intended to aid in establishing limits for exposure in both occupational and community settings. The study helps to determine specific occupational exam requirements, personal protective equipment requirements and methods for monitoring exposure. Additionally, by correlating ambient exposure measures with health and performance outcomes, we hope to use the data obtained from this study to extrapolate the extent of community risks associated with ubiquitous, low-level jet fuel exposure.

The study was conducted in conjunction with Texas Tech University, the University of Cincinnati, and the Oregon Health Sciences University. Additional collaborators include the University of Texas, the University of North Carolina, Johns Hopkins University, the US Navy Toxicology Laboratory at Wright-Patterson AFB, OH, the NIOSH, and EPA/NERL.

The study's purpose was to assess the influence of acute exposure to jet fuel on the health, safety and operational capability of the Air Force population and gain insight into the risk posed by JP8 on the general local population.

The specific aims were to:

a) Compare exposure levels of a selected worst-case exposed cohort to the generally unexposed base workforce.

b) Determine level of body burden of jet fuel within each exposure group.

c) Analyze biological specimens from each subject group for jet fuel-linked specific biomarkers of exposure and effect.

d) Perform an epidemiology analysis of each subject group.

e) Assess the impact of JP8 exposure on performance and health outcomes.

f) Perform a risk analysis for environmental and occupational communities based on collected sample data.

The primary hypotheses addressed through this study are the following:

Is exposure to JP8 detrimental to the health and safety of flightline workers? Does a low-level ambient exposure to jet fuel have an adverse impact on the general community at an Air Force installation?

The study was conducted at multiple Air Force installations. Dyess AFB, TX, served as the beta test site for participant selection, specimen collection, and exposure assessment. The lessons learned from the Dyess AFB beta test allowed the JP8 Research Team to improve data collection processes and study logistics, thus reducing the operational study impact at other Air Force bases involved in the study. Data was collected at the following sites: Davis Monthan AFB, AZ, Seymour Johnson AFB, NC, Langley AFB, VA, Pope AFB, NC, Little Rock AFB, AR, and Hurlbert Field, FL. Specimens and data collected from these locations were analyzed at established laboratory facilities operated by the universities and government agencies involved in the study.

General Methods

The Risk Assessment of Acute Exposure to Jet Fuel study measured JP8 exposures in an operational environment and assessed the impact of exposure on the performance and health of those enrolled in the study. JP8 exposure was measured both externally in the environment immediately surrounding enrolled workers and internally through the use of several body burden measures. The impact of exposure was evaluated using a series of neurological, hormonal and immunological measures. Cytotoxic and genotoxic effects of JP8 exposure were also evaluated. The activity of Glutathione-S-Transferase (G-S-T), a gene-regulated enzyme associated with increased susceptibility to multiple oxidative stressors including jet fuel and linked to adverse health outcomes, was also measured. Self-reported health problems, health care visit frequency, and early indicators of liver and kidney damage were investigated as part of the study.

Study Logistics

The Jet Fuel Research Team, a group of approximately 30 researchers from six academic institutions, two government agencies and two military services, traveled to six Air Force bases in the continental United States to conduct the study. Visits were coordinated in advance to obtain Commander permission to conduct the study. Commanders were briefed in person or by video teleconference prior to the visit to provide information on the rationale for the study, the study goals, milestones to be accomplished during the visit, and the logistics associated with conducting the study on their base. The study was conducted during a two-week period at each study site. One Air Force base was visited every month between April and September 2000. A beta test was conducted prior to the initial site visit to test the logistics of moving people and equipment and synchronizing the timing of multiple specimen collections and testing applications.

Study Subject Recruitment:

Recruitment at each study site was initiated prior to study team arrival and continued throughout the first week of the visit. Subjects were recruited for the study through several vehicles. Since the primary exposure group for the study were workers from shops where contact with jet fuel routinely occurs, the supervisors of such shops as Aircraft Fuel Systems Maintenance, Fuels Transportation, and Fuels Specialty were directly contacted to gain support for the study and solicit volunteers. Members of the fuels community, particularly aircraft fuel systems maintenance personnel, supervisors and commanders, showed high interest in the project and large numbers of workers from these shops volunteered for the study.

Additional recruitment efforts consisted of briefings at Commanders Calls, and informational press releases and solicitation advertisements in local military installation newspapers. At some study locations, First Sergeants were contacted to help gain support for the study. A financial incentive of $50.00 was provided by Texas Tech University to compensate subjects for their participation outside of regular duty hours. Those who completed all requested tests and provided all requested specimens received $50.00. Any subject who dropped out prior to completing the study received $10.00.

Recruitment was successful at all study locations. At several locations, volunteers were turned away after a sufficient number of subjects was achieved. While the study actively recruited females, few women work in jobs where jet fuel exposure occurs. The unexposed to exposed ratio for women was increased 2 : 1 as originally planned 4 : 1 in an attempt to improve the ability to detect differences in effect.

Study Subject Enrollment:

Two groups of airmen were enrolled. Those designated as **JP8 exposed** consisted of active duty Air Force personnel who routinely worked with or are exposed to JP8 in the performance of their duties. Most exposed volunteers worked in Aircraft Fuel Cell Maintenance shops. These workers routinely performed maintenance activities requiring entry into aircraft fuel tanks. Other exposed volunteers worked in either the Fuels Specialty or Fuels Transportation shops. In order to qualify for the study, exposed volunteers were required to have least 9 months of persistent exposure to jet fuel (such as fuel tank entry at least one hour twice weekly).

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All volunteers were informed of the nature of the study and the potential risks associated with participation. By groups of approximately 50, volunteers were given a 30 to 45 minute briefing by an occupational medicine physician. The script used for the briefing had undergone extensive review and testing prior to employment. Groups of researchers and potential volunteers were asked to comment on the briefing during the beta-testing portion of the study. In addition, volunteers at each study site were asked to comment of the acceptability and completeness of the briefing. Without exception, the members of the JP8 study team and study volunteers considered the standardized briefing highly acceptable.

In addition to the briefing, study volunteers were asked to complete a questionnaire designed to obtain information on specific criteria that could disqualify them from participating in the study. Exclusion criteria consisted of conditions that would impact the validity of either study effects or exposure measures. Those criteria were:

1. Alcohol use within 24 hours prior to entering the study period
2. Injury requiring medical attention within the last 6 months
3. History of melanoma
4. History of congenital night blindness
5. History of lung or ovarian cancer
6. History of adult cerebral vascular accident
7. History of diabetes
8. History of scoliosis
9. Major visual impairment
10. Clinical diagnosis of seizures
11. On medical profile
12. Pregnancy
13. Currently taking any medications determined by an occupational medicine physician to be disqualifying. Such medications included:

    a) Hypertension medication
    b) Antacids or medication for heartburn
    c) Diet pills or other stimulants
    d) Tranquilizers or muscle relaxants
    e) Antidepressive medication
    f) Psychotherapeutic medication
    g) Large doses of megavitamins containing high levels of antioxidants

Each volunteer underwent a personal interview with either an occupational or preventive medicine board certified physician where the volunteer's completed questionnaire was reviewed and specific volunteer questions were addressed. After the physician determined the volunteer was eligible to participate in the study, the volunteer and the physician completed an informed consent document. The new enrollee was then given appointments for study testing. Each enrollee was assigned a unique study code consisting of the first three letters of his or her assigned Air Force base, e.g. Pope AFB = POP, and a randomly generated number between 1000 and 9999. A reference log consisting of enrollee's social security number, subject code, and exposure group classification was created, maintained, and safeguarded by the occupational medicine physician. All researchers throughout specimen collection, performance testing, and data analysis phases of the study used the study codes for recording information relative to the enrollee. The use of study codes helped maintain subject confidentiality and assisted in blinding researchers to enrollee exposure status. At the end of the study, the reference log was forwarded to Texas Tech University for permanent storage.

Specimen and Data Collection

In most cases, all exposure measurements and performance/health effects testing were conducted during one subject's workday. Enrollees typically reported for testing on an appointed morning. Each subject was asked about their alcohol and tobacco consumption during the 24 hours prior to testing and whether he or she was experiencing cold or allergy symptoms. Those with cold or allergy symptoms and those who had consumed alcohol within 24 hours were rescheduled to another day whenever possible. Tobacco use was recorded.

From those who met morning test entry parameters, specimens of blood, breath, urine and epidermal skin were collected. Samples of the cells from the interior of the cheek were also collected for later testing. The enrollees completed a series of tests designed to measure various neurological parameters. Prior to returning to work, each volunteer was fitted with equipment designed to collect samples of the air within their breathing zones during the work period. Enrollees were also fitted with equipment designed to measure their heart rate and core body temperature throughout the workday.

After undergoing morning testing, the enrollees returned to their usual workplace and performed routine duties for a period of at least 4 hours. During the time the enrollees were at work, members of the research team collected environmental measures. While most of the environmental samples were collected in or near the Aircraft Fuel System Maintenance Shop, a representative number of samples were gathered from other locations to ensure that those enrollees categorized as unexposed were, in fact, unexposed to jet fuel or similar chemicals.

In the afternoon, enrollees returned to the study site where environmental and vital status monitoring equipment was removed. Post-workday specimens of blood, breath, urine and epidermis were collected and a series of tests similar to those conducted in the morning were repeated. Questionnaires were applied to the enrollees to obtain information regarding the level of mental and physical exertion experienced during the day and details of the individual's activities throughout the work period. Questionnaires designed to capture information on self-reported symptoms, lifestyle risk (such as smoking and drinking), and the use of personal protective equipment were also applied.

After completing all specimen collection and testing, the enrollees received their study stipend and were released. At the end of the week, the researchers departed the base. Of note, the researchers collected information on the exact time of day each specimen was collected and each test was performed for each enrollee using a subject-time-series log. These time-series data were made available to all investigators to aid in analysis.

One test, the electroretinogram (ERG), was not accomplished during the typical data collection week. The ERG, a method of measuring retinal function, was administered to a subset of enrollees during the week prior to the normal data collection period. Studies in animals chronically-exposed to JP8 have shown selective cellular damage to cells located in the retina and cerebellum. Since any retinal changes detectable by the ERG would be the result of chronic

exposure, repeat ERG testing (pre and post work period) was unnecessary. Approximately 20 subjects at each base were selected to complete the ERG.

Exposure measures:

The JP8 exposure measures conducted as part of the JP8 study are briefly discussed below. A more detailed explanation of each exposure measure is provided in the abstracts included in this report.

Biological measures:

Blood: Each subject submitted two 40-ml blood specimens -- one specimen during the morning test period and one in the afternoon. Trained phlebotomists from the Air Force Research Laboratory (AFRL) at Wright-Patterson AFB collected all blood specimens. Each blood specimen was divided into three aliquots. Texas Tech University conducted quantitative analysis for the enzyme Glutathione-S-Tranferase (G-S-T) in blood. Researchers from Brooks AFB, in collaboration with researchers from the EPA, conducted analysis for JP8 markers. Scientists from the University of North Carolina analyzed blood specimens for metabolites of benzene and naphthalene. NIOSH and Navy collaborating scientists conducted additional biomarker analyses. A small amount of residual blood from each subject was provided to AFRL for physiologically-based pharmacokinetic (PBPK) modeling of jet fuel metabolism.

Urine: Urine samples collected prior to and after the sampling period were divided into two aliquots. Researchers from the University of North Carolina analyzed urine for the presence of metabolites for benzene and naphthalene. NIOSH conducted analysis of urine samples for the presence of renal biomarkers of exposure.

Breath: Three breath samples were typically collected before and after the work period. Using devices called SUMMA canisters, a scientist from the EPA collected breath samples from selected enrollees and analyzed the specimens for the presence of JP8 markers. Breathe samples, collected using a 75-ml glass bulb collection device, were processed by University of North Carolina scientists to identify the presence of benzene and naphthalene. Using a third breath collection method, a researcher from Johns Hopkins University obtained pre- and post-work samples from selected enrollees and performed an analysis to quantify the amount of JP8 constituents contained in each specimen.

Skin Exposure Sampling: Epidermal specimens were collected prior to and following the work period using a dermal taping method. The skin specimens were analyzed for the presence of naphthalene by researchers from the University of North Carolina.

Body Temperature Monitoring: Internal body temperature, a potential confounding variable in the association between jet fuel constituent metabolism and performance/health measures, was monitored during the enrollee's work period. Selected subjects were asked to swallow a small pill-like sensor. The device provided continuous monitoring of body core temperature during the enrollee's work period. Other enrollees were asked to wear an aural or skin temperature probe. All enrollees wore Polar Band heart rate monitors around the chest area, and activity sensors on the wrist.

Performance/Health Measures:

Enrollees were asked to submit to a series of performance and health effects measures. Tests included the Global Assessment System for Humans/Behavioral Assessment and Research System (GASH/BARS), the Postural Sway Test, the Eye Blink Conditioned Response Test and the Electroretinogram (ERG). Subjects were also asked complete an electronically-administered questionnaire. Medical records were reviewed for pertinent health events occurring during the preceding year.

Global Assessment System for Humans (GASH) / Behavioral Assessment and Research System (BARS): The GASH/BARS system consists of a series of computer-based neuro-behavioral tests designed to measure motivation, response speed, coordination, grip strength, complex mental functioning, memory, and attention. Subjects completed the GASH/BARS test series prior to and after the work period. Data from the subjects' Air Force Qualifying Test (AFQT) were also obtained from the Air Force Personnel Center and used to support the GASH/BARS analysis. AFQT exam scores were coded using subject codes to protect subject confidentiality and ensure study blinding.

Electroretinogram (ERG): The ERG is a device designed to measure the electrical response of the eye to brief, high intensity flashes. In this study, the ERG was used to determine the association between JP8 exposure and retinal Mueller cell function. Subjects who volunteered for this protocol underwent an ERG as part of their evaluation. Since the hypothesized retinal changes are associated with chronic JP8 exposure, the ERG procedure was accomplished only once on the enrollee selected. In addition to comparisons between the exposed and unexposed groups, ERG results were compared with normative data.

Postural Sway: A team of researchers from the University of Cincinnati conducted a series of tests to assess the enrollee's balance. During the test, subjects were asked to perform a series of procedures while standing on a platform designed to measure changes in balance. The procedures included standing on the platform alone and with a foam pad between the platform and enrollee's feet while performing a series of procedures with their eyes open or closed. Each enrollee also answered a short list of questions prior to postural sway testing.

Eye Blink Conditioned Response (ECR): The eye blink response is a reflex that can be classically conditioned. The ECR is considered a sensitive measure of more global issues of brain functioning, and is appropriate for assessing robust and/or subtle changes in neural processing that one might expect from repeated exposure to jet fuel vapors. Enrollees completed the ECR during pre- and post-work periods. Navy technicians conducted this procedure on selected enrollees. The Navy Neurotoxicology Group at Wright-Patterson AFB analyzed the results of ECR tests.

Risk Factor Questionnaire. Each subject volunteer completed a series of questions designed to assess self-reported symptoms, and exposure to potentially confounding factors, such as alcohol and tobacco. Questions regarding hobbies and work-shift history were also addressed.

Subjects also completed a series of standardized questions from a copyrighted questionnaire termed the SF-36. Both questionnaires were administered electronically after completion of the GASH/BARS.

Medical Records Review: Epidemiologists from Texas Tech University and AFIERA reviewed the medical records of those enrolled in the study. The epidemiologists recorded health care events occurring during the year prior to the study period using broad disease categories. Associations between health care event frequency and JP8 exposure were tested using these data.

Analysis:

Data collection.

Members of the JP8 research team collected all exposure specimens and outcome data. For the most part, the researchers associated with specific sub-protocols (such as the Postural Sway Test) included in the overall JP8 study were responsible for applying tests, analyzing specimens and collecting data specific to their sub-protocols. The exceptions to this rule were time-series logs, study eligibility, exertion and daily activity questionnaires, and blood specimen collection. The blood specimens were collected by AFRL phlebotomists, divided into aliquots and provided to other researchers. The questionnaires and logs were collected by AFIERA personnel, entered into spreadsheets and provided to all researchers to aid in their analyses.

In total, 339 Air Force active duty members were enrolled in the study. Of those enrolled, 324 completed all required tests and submitted all required specimens. Some enrollees were not able to complete the entire study due to unavoidable circumstances. Eight enrollees completed only the ERG, 3 only completed a questionnaire, 2 completed only the ERG and a questionnaire, and 2 enrollees completed all but one or two of the required tests.

Initial Exposure Classification:

Using information provided by the enrollees and exposure stratification assignment at the time of enrollment, those enrolled in the study were categorized into one of four groups. The exposure categories were based on the probability of JP8 exposure in the completion of normal operational duties. The exposure categories and decision tree for enrollee categorization are listed below.

Category	Criteria	Result
HI	Classified as exposed by study bioenvironmental engineer?	YES
	AND BY SELF REPORT	
	Does your current job routinely bring you into physical contact with jet fuel?	YES
	What is your primary AFSC?	2A6X4
	Does working in your primary AFSC bring you into physical contact with jet fuel?	YES
	Age under 35	YES

HI MOD	Classified as exposed by study bioenvironmental engineer?		YES
	BY SELF REPORT		
	Does your current job routinely bring you into physical contact with jet fuel?		YES
	Does working in your primary AFSC bring you into physical contact with jet fuel?		YES
	AND		
	What is your primary AFSC?		2FOXX 2T3XX 2E4XX
	OR		
	Classified as exposed by study bioenvironmental engineer?		YES
	BY SELF REPORT		
	Does your current job routinely bring you into physical contact with jet fuel?		YES/NO
	Does working in your primary AFSC bring you into physical contact with jet fuel?		YES/NO
	MUST ANSWER NO TO ONE OF THE ABOVE QUESTIONS		
	AND		
	What is your primary AFSC?		2A6X4
	OR		
	Primary AFSC = 2A6X4 and Age 35+		
MOD	Classified as exposed by study bioenvironmental engineer?		NO
	BY SELF REPORT		
	Does your current job routinely bring you into physical contact with jet fuel?		YES/NO
	Does working in your primary AFSC bring you into physical contact with jet fuel?		YES/NO
	MUST ANSWER YES TO ONE OF THE ABOVE		
	AND		
	What is your primary AFSC?	ANY BUT	2A6X4 2FOXX 2T3XX 2E4XX
LOW	Classified as exposed by study bioenvironmental engineer?		NO
	BY SELF REPORT		
	Does your current job routinely bring you into physical contact with jet fuel?		NO
	Does working in your primary AFSC bring you into physical contact with jet fuel?		NO
	AND		
	What is your primary AFSC?	ANY BUT	2A6X4 2FOXX 2T3XX 2E4XX

The categorization scheme was developed and employed for two reasons. First, as pointed out in the abstracts following this section, processing specimens is time-consuming. Six months after data collection, some exposure data, particularly measures of JP8 in blood, remained unavailable to researchers. Measures of JP8 effects, particularly neurological test results, were however available for analysis shortly after the end of the data collection phase. The categorization scheme was employed to allow those measuring JP8 effects to obtain a preliminary assessment of degree to which JP8 impacted human performance. The second reason for employing the categorization scheme relates to chronic measure of effects. Effects measures, such as the ERG, medical visit history and self-reported symptoms, are not necessarily related to acute JP8 exposure, but may be influenced by chronic exposure to jet fuel. Since the

categorization scheme assesses the probability of occupational JP8 exposure, it serves well as a means for stratifying enrollees based on chronic exposure.

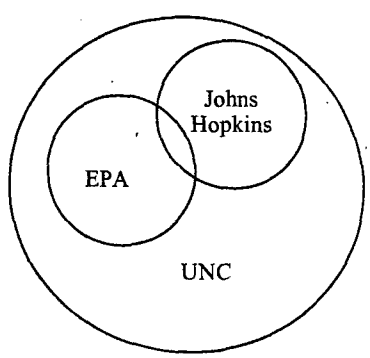
While the categorization scheme provides a method for determining JP8 exposure, body burden measures employed in this study provide a direct method for quantifying exposure. Direct body burden measures should be far superior to categorization in assessing acute exposure. Three strategies were employed to hasten the availability of JP8 body burden measures.

Strategy One. Prioritization of Specimen Processing

While the processing of all samples is needed to achieve sufficient sample size to determine statistically significant differences in health and performance effects based on JP8 body burden, investigators could estimate the strength of JP8 effects by conducting analyses using a representative sample of the data. To provide this sample as quickly as possible, a stratified random sample of the study enrollees was selected and all investigators were notified to process specimens from these enrollees first. A weighted sample consisted of 110 enrollees chosen at random after stratifying all enrollees by exposure category. Research may refer to this prioritized sample in the abstracts that follow.

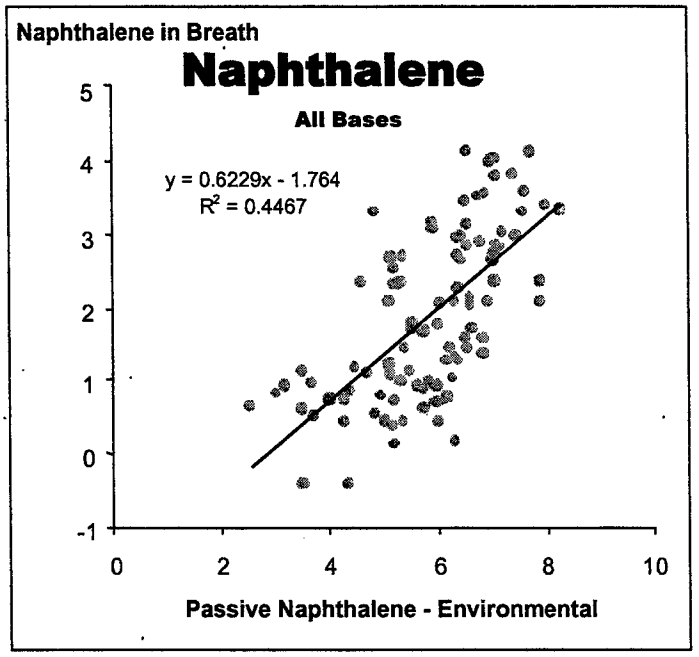
Strategy Two. Selection of a Single Continuous Measure of Exposure

In January 2001, a group of JP8 researchers, including the three researchers responsible for measuring JP8 in breath specimens, met to consider whether one single breath measure could sufficiently provide a continuous measure of JP8 body burden for use by other researchers involved in the study. The group reviewed available breath data and found reasonable agreement between the three breath measures with correlation coefficients in the 0.7 to 0.8 range. As graphically represented below, breath measures via EPA and John Hopkins methodologies though highly correlated, were not available for all subjects. The breath measures provided by the University of North Carolina provided data on two constituents of JP8: benzene and naphthalene. Though not as highly correlated as the EPA and Johns Hopkins data, the naphthalene data was generally in agreement with the other breath measures and was available on nearly all enrollees. The exposure measurement team of researchers agreed to provide naphalene measurement data to all collaborating researchers for use in assessing the impact of JP8 acute exposure. The abstracts that follow will, in some cases, refer to these measures in their analyses.



Strategy Three:

Preliminary analysis of JP8 exposure using post-workday naphthalene breath samples failed to demonstrate any association between exposure and the three primary neurologic tests used in the study. Not only was no association demonstrated, the findings noted during analysis using the exposure categories described under Strategy One were lost. Further, an analysis performed by Egeghy and graphically displayed below showed weak correlations (R^2 = -0.44) between post-workday naphthalene specimens and subject-specific environmental naphthalene samples.

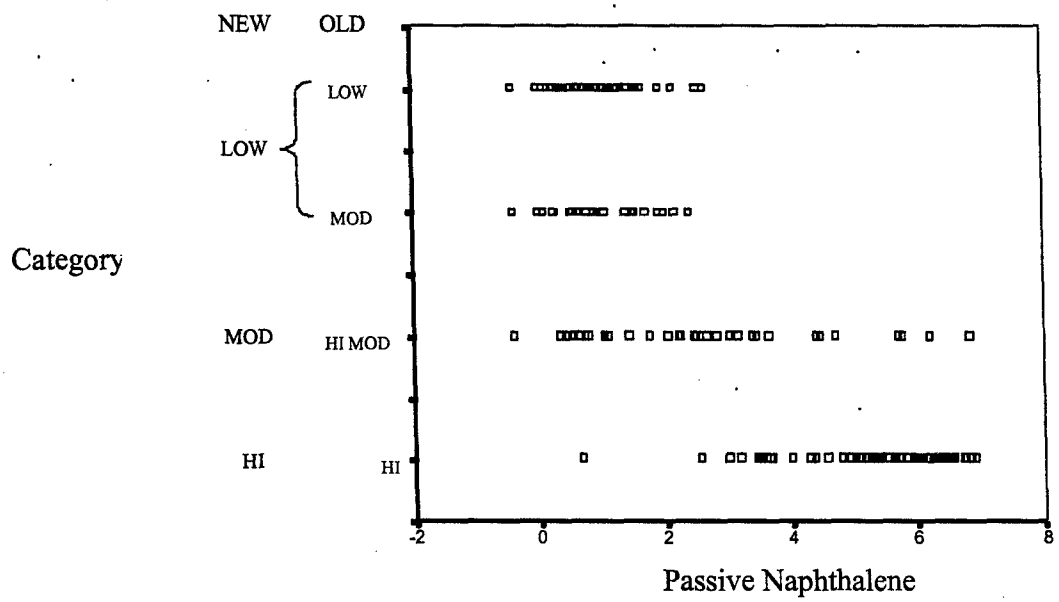


Egeghy, University of North Carolina

Based on these findings, the research team investigated the correlation between environmental naphthalene samples and the previously developed exposure categories. Initial analysis showed much higher correlations (R^2 = -0.83). Investigation of outliers revealed the highest environmental naphthalene measures among the LOW exposure category involved subjects tested on Monday and Tuesday at Davis Monthan AFB. Study notes showed investigators were concerned about the possibility of secondary exposure among LOW exposed subjects during post-sampling periods on Monday and Tuesday. LOW exposed subjects returned to the sample collection site at the same time as HI exposed subjects. Further, because of the building design, a strong fuel odor was reported to the research team. Measures were taken to prevent secondary exposure during the following days at Davis Monthan AFB and at subsequent study locations. Based on these findings, the LOW exposed subjects from Monday and Tuesday at Davis Monthan AFB were eliminated from the preliminary analyses.

Outlier analysis also revealed one subject LAN9356 was miscoded. The subject, originally coded as moderately exposed (MOD), actually worked in Aircraft Fuel System Repair

Shop and handled fuel-soaked fire suppression foam on the day of the test. After the subject's exposure code was changed and the Davis Monthan ABF LOW-exposed subjects from Monday and Tuesday were removed from the dataset, correlation coefficients improved to $R^2 = -0.86$. Since the LOW and MOD categories were indistinguishable with respect to environmental naphthalene measures, the categories were collapsed into one category term LOW. The HI MOD category was renamed MOD and the HI category remained unchanged. Researchers may refer to this new categorization scheme in the abstracts included in the report.



Risk Characterization

The full characterization of JP8 health risks, to be conducted by Texas Tech University, will take into account exposure measures of JP8 and measures of effect. The risk characterization will attempt to determine the association between the various measures of effect used in this study and assess the overall impact, by JP8 dose, on workers exposed to the fuel. Details regarding the JP8 risk analysis are provided at the conclusion of this report.

Summary for Website - General Methods

The Risk Assessment of Acute Exposure to Jet Fuel study measured JP8 exposures in an operational environment and assessed the impact of exposure on the performance and health of those enrolled in the study. JP8 exposure was measured both externally in the environment immediately surrounding enrolled workers and internally through the use of several body burden measures. The impact of exposure was evaluated using a series of neurological, hormonal and immunological measures. Cytotoxic and genotoxic effects of JP8 exposure were also evaluated. The activity of Glutathione-S-Transferase (G-S-T), a gene-regulated enzyme associated with increased susceptibility to multiple oxidative stressors including jet fuel and linked to adverse health outcomes, was also measured. Self-reported health problems, health care visit frequency, and early indicators of liver and kidney damage were investigated as part of the study.

> The Jet Fuel Research Team, a group of approximately 30 researchers from six academic institutions, two government agencies and two military services, traveled to six Air Force bases in the continental United States to conduct the study.

Two groups of airmen were enrolled. Those designated as **JP8 exposed** consisted of active duty Air Force personnel who routinely worked with or are exposed to JP8 in the performance of their duties. Most exposed volunteers worked in Aircraft Fuel Cell Maintenance shops. These workers routinely performed maintenance activities requiring entry into aircraft fuel tanks. Other exposed volunteers worked in either the Fuels Specialty or Fuels Transportation shops. In order to qualify for the study, exposed volunteers were required to have at least 9 months of persistent exposure to jet fuel (such as fuel tank entry at least one hour twice weekly).

Unexposed volunteers were intended to represent the population of active duty Air Force personnel assigned to the Air Force installation where the study was being conducted. They consisted of active duty personnel assigned to the same Air Force installation as the JP8 exposed volunteers, but who do not have routine contact with JP8 or other fuels during the performance of their duty. A wide variety of job classifications were represented in the unexposed group.

Before work, specimens of blood, breath, urine and epidermal skin were collected from each enrollee and a series of tests designed to measure various neurological parameters were completed. Each volunteer was also fitted with equipment designed to collect samples of the air within their breathing zones and measure heart rate and core body temperature throughout the workday. At the end of a four-hour work period the enrollees returned to their usual workplace and performed routine duties for a period of at least 4 hours. During the work period, members of the research team collected environmental measures. Questionnaires were applied to obtain information regarding the level of mental and physical exertion experienced during the day and details of the individual's activities throughout the work period. Questionnaires designed to capture information on self-reported symptoms, lifestyle risk (such as smoking and drinking), and the use of personal protective equipment were also administered.

Biological measures:

Blood: Each subject submitted two 40-ml blood specimens, which were divided into three aliquots. Specimens were analyzed for Glutathione-S-Transferase (G-S-T) enzyme activity, the presence of JP8 constituents, early indicators of liver and kidney damage, hormone levels, immunologic function and other biomarker of exposure and effect. Blood specimens were also used in physiologically-based pharmacokinetic (PBPK) modeling of jet fuel metabolism.

Urine: Urine samples collected prior to and after the sampling period were divided into two aliquot and analyzed for the presence of benzene and naphthalene metabolites and renal biomarkers of exposure.

Breath: Three breath samples were typically collected before and after the work period. They were analyzed for the presence of JP8 constituents and metabolites using three separate methodologies.

Skin Exposure Sampling: Epidermal specimens were collected prior to and following the work period using a dermal taping method and analyzed for the presence of naphthalene.

Performance/Health Measures:

Enrollees were asked to complete a series of performance and health effects measures before and after a four-hour work period. Tests included the Global Assessment System for Humans/Behavioral Assessment and Research System (GASH/BARS), the Postural Sway Test, the Eye Blink Conditioned Response Test and the Electroretinogram (ERG). Subjects were also asked to complete an electronically-administered questionnaire. Medical records were reviewed for pertinent health events occurring during the preceding year.

Global Assessment System for Humans (GASH) / Behavioral Assessment and Research System (BARS): The GASH/BARS system consists of a series of computer-based neuro-behavioral tests designed to measure motivation, response speed, coordination, grip strength, complex mental functioning, memory, and attention.

Electroretinogram (ERG): The ERG, a device designed to measure the electrical response of the eye to brief, high intensity flashes was used to determine the association between JP8 exposure and retinal Mueller cell function. In addition to comparisons between the exposed and unexposed groups, ERG results were compared with normative data.

Postural Sway: Each enrollee underwent a balance test prior to and after the work period. During the test, subjects were asked to perform a series of procedures while standing on a platform designed to measure changes in balance. The procedures included standing on the platform alone and with a foam pad between the platform and enrollee's feet while performing a series of procedures with their eyes open or closed. Each enrollee also answered a short list of questions prior to postural sway testing.

Eye Blink Conditioned Response (ECR): The ECR is considered a sensitive measure of more global issues of brain functioning, and can detect subtle changes in neural processing. Enrollees completed the ECR during before and after the 4-hour work period.

Risk Factor Questionnaire. Each enrollee completed a series of questions designed to assess self-reported symptoms, and exposure to potentially confounding factors, such as alcohol and tobacco. Questions regarding hobbies and work-shift history were also addressed. Subjects also completed a series of standardized questions from a copyrighted questionnaire termed the SF-36.

Medical Records Review: Epidemiologists from Texas Tech University and AFIERA reviewed the medical records of those enrolled in the study. The epidemiologists recorded health care events occurring during the year prior to the study period using broad disease categories. Associations between health care event frequency and JP8 exposure were tested using these data.

Analysis

Members of the JP8 research team collected all exposure specimens and outcome data. For the most part, the researchers associated with specific sub-protocols (such as the Postural Sway Test) were responsible for applying tests, analyzing specimens and collecting data specific to their sub-protocols. The exceptions to this rule were time-series data logs, study eligibility, exertion and daily activity questionnaires, and blood specimens. The blood specimens were collected by phlebotomists, divided into aliquots and provided to other researchers. The questionnaires and logs were provided to all researchers to aid in their analyses.

In total, 339 Air Force active duty members were enrolled in the study. Of those enrolled, 324 completed all required tests and submitted all required specimens.

Initial Exposure Classification:

Using information provided by the enrollees and exposure stratification assignment at the time of enrollment, those enrolled in the study were categorized into one of four groups based on the probability of JP8 exposure in the completion of normal operational duties. This categorization scheme was later amended as additional exposure data became available. These categories were used to a varying degree by collaboration researchers.

Risk Characterization

The full characterization of JP8 health risks, to be conducted by Texas Tech University, will take into account exposure measures of JP8 and measures of effect. The risk characterization will attempt to determine the association between the various measures of effect used in this study and assess the overall impact, by JP8 dose, on workers exposed to the fuel. Details regarding the JP8 risk analysis are provided at the conclusion of this report.

Enrollment Results

Potential subjects for the Risk Assessment of Acute Exposure to Jet Fuel study were solicited from six Air Force bases in the continental United States. Of the approximately 450 candidates who responded to recruitment efforts, 394 received the study briefing and completed the exclusion criteria questionnaire. From these candidates, 339 subjects met minimal enrollment criteria and entered the study. Enrollment percentages by study site are listed below.

Study Site	Number Considered	Number Enrolled	Percent Enrolled
Davis Monthan	74	65	87.8
Seymour Johnson	70	49	70.0
Langley	66	59	89.4
Pope	56	60	93.3
Little Rock	64	49	76.6
Hurlbert Field	64	57	89.1
Total	394	339	86.0

Reasons for ineligibility included:

Preexisting medical condition	20
Contraindicated prescription drugs or over-the-counter vitamins	17
Recent surgery	3
Not enough time-on-station	3
TDY during the week of the study	4
On quarters or profile	2
Pregnant	2
Candidate opted out	4
TOTAL	**55**

Of the 284 males and 55 females that began the study, 15 withdrew before the study was completed. Eight completed only the electroretinograph (ERG) test, 3 completed only the Risk Factor Questionnaire (RFQ), and 2 completed both the ERG and RFQ before dropping out. Two additional subjects withdrew with after completing nearly all parts of the study. Most of the withdrawals (7) were due to a tropical storm that arrived at Hurlbert Field the week of the study. The storm forced researchers to cancel the final data collection day of the study.

Details regarding the enrolled subjects are included in the table below. A total of 284 men and 55 women were enrolled. Subject ages ranged from 18 to 44 years, with an average age of 26.1 and a median age of 24 years. The exposure categories used in the table below represent the revised categorization discussed in the General Methods section. The LOW categories include subjects with no or rare exposure to JP8. Subjects in the MOD category do not have daily exposure to JP8, but may periodically perform tasks requiring fuel exposure. The HI category includes only personnel assigned to Aircraft Fuels System Maintenance shops.

Risk Assessment of Acute Exposure to Jet Fuel
Personal Characteristics

	Males = 284			Females = 55		
	Exposure Status			Exposure Status		
	HI	MOD	LOW	HI	MOD	LOW
Age (mean)	24.6	26.8	27.6	22.6	33.8	24.8
Age by Group (%)						
Under 20	3.5	2.6	3.1	20.0	0.0	5.0
20 to 24	57.4	48.7	42.3	50.0	0.0	55.0
25 to 29	27.8	20.5	20.0	20.0	20.0	25.0
30 to 34	4.3	7.7	15.4	0.0	20.0	7.5
35 to 39	5.2	17.9	13.8	0.0	60.0	5.0
40 and Over	1.7	2.6	5.4	0.0	0.0	2.5
Right Handed (%)	83.5	76.9	83.8	100.0	80.0	95.0
Caucasian (%)	80.0	74.4	72.8	80.0	100.0	70.0
Height in inches (mean)	70.5	70.5	70.7	66.2	66.2	65.0
Weight in pounds (mean)	178.0	182.3	186.7	143.7	151.4	145.0
Body Mass Index (mean)	28.4	27.7	27.2	31	29.7	29.5
Smoke at least 1/4 Pack/Day (%)	43.9	47.4	32.3	50.0	60.0	30.8
Alcohol Users (%)	61.7	66.7	74.2	75.0	100.0	64.1
Daily Caffeine Users (%)	51.9	66.7	53.5	75.0	60.0	28.2
Eat Processed Meats > 1 Time/Week (%)	43.5	35.9	40.3	37.5	20.0	28.9
Months of the Job	53.8	49.2	57.5	31.9	34.6	30.55
Months on Current Base	33.3	28.5	33.2	19.8	43.2	26.9
Engage in Physical Activity 1 to 2 Times Per Week (%)	62.5	66.7	60.7	100.0	100.0	61.1
Work 8 to 10 Hours Per Day at Job (%)	94.4	84.6	85.9	75.0	100.0	89.7
A Great Deal of Physical Work is Required as Part of Job (%)	13.9	5.1	7.0	25.0	0.0	0.0
Physical Exertion Score (mean)	10.7	9.3	7.0	10.2	8.4	5.6
Mental Exertion Score (mean)	3.9	3.5	4.0	3.5	4.0	3.9
Find Job Very Stressful (%)	26.2	5.3	19.5	12.5	20.0	5.1

Exposure categories were reasonably comparable with respect to right-handedness, race, height, weight and body mass index. The percentage of smokers, alcohol, caffeine, and processed meat users is approximately the same in all categories. Subjects report approximately the same number of hours worked in a week and engage in same amount of physical activity off duty. While subjects in the highest JP8 exposure categories were, on average, younger than those in the other exposure categories, the biological significance of these age differences is questionable. No differences are seen in the months on the base and in the current job. In two different physical exertion measures, however, the amount of physical work required to perform duties associated with their job, is significantly greater (P-value <0.001) for those in the HI category. No differences are seen in the mental exertion required. Male subjects in the HI category are more likely to report a great deal of stress associated with their job. Due to much smaller sample size, comparisons of personal characteristics among females are less stable than those of males.

JP8 exposure results and the impact of JP8 exposure on various performance tests and health outcome measures are reported in the abstract found in this report.

Quantification of Dermal Exposure to Jet Fuel
Risk Assessment of Acute Exposure to Jet Fuel

Introduction

Dermal exposure to hazardous chemicals in the workplace is an important occupational hygiene issue and a growing field of interest to health professionals in both occupational hygiene and dermatology. In this study, acute dermal exposure to JP8 was investigated among Air Force fuel cell maintenance workers. Because one of the main routes of exposure to the jet fuel is through skin, it is important to assess the potential degree of dermal exposure and the possible implications of adverse health effects. We used a validated non-invasive tape-stripping technique to determine JP8 contamination in the skin. This technique removes the upper layers of the stratum corneum (dead skin cell layers) using successive tape stripping with an adhesive tape, which allows for determination of the quantity of JP8 retained in the skin following exposure. We measured naphthalene as a marker for jet fuel exposure. Using this technique, in conjunction with biological monitoring, we aim to relate the amount of contamination in the skin to the amount actually absorbed into and through the skin and consequently made available for the uptake by the body (total body dose).

Methods

Tape Stripping

Three sites per subject were chosen for the tape-stripping based on regions of the body that were exposed to jet fuel. Each site was tape stripped 3 times in succession, using the Cover-Roll™ adhesive tape, cut to size 2.5 cm × 4 cm. The backing was removed prior to application to the skin and the tape pressed to the skin with a constant pressure. The tape was allowed to remain on the skin for 2 min and removed by using clean forceps and peeling at a 45° angle. The tape was folded the adhesive side in and placed into a labeled 20 ml scintillation vial containing 5 ml nanograde acetone (extraction solvent) and 20 µl of 25 µg/ml naphthalene-d_8 for gas chromatography/mass spectrometry (GC/MS) analysis.

Gas Chromatography/Mass Spectrometry Analysis

After tape-stripping, the scintillation vials were sealed and shipped to UNC, where they were stored at 4°C until analysis. Samples were acclimated to room temperature and placed on a rotary shaker for 30 min at 250 rpm. The adhesive tape was removed from each vial using clean forceps and any remaining solution was squeezed from the tape back into the vial. Gloves were changed and the forceps were rinsed between each sample to avoid cross-contamination. Samples were then concentrated from 5 ml to 0.5 ml using compressed nitrogen. The remaining solution was transferred to 2 ml amber autoinjector vial for GC/MS analysis. All samples were analyzed using a ThermoQuest TraceGC in series with a Finnigan Polaris Q mass spectrometer.

The first tape strips from each subject were analyzed first. If naphthalene amount found in the first tape strip was above the limit of detection (6 pg/µl injected or 3 ng per tape strip), analysis of the second and third tape strips from these sampling sites was conducted. Field blank samples as well as laboratory media blank samples were analyzed concordantly and results were field blank corrected.

Status

GC/MS analyses are complete for all samples (subject N = 126; sample n = 753), with the exception of the second or third successive tape strips where analysis was not warranted, as determined by analysis of the first tape strips. Data has been input for naphthalene mass by sample ID, and general descriptive statistics have been performed.

Findings

The overall mean, median, and range of naphthalene exposures for all subjects were 35.7 ng, 15.0 ng, and <LOD – 25287 ng per tape strip, respectively. The mean, median, and standard deviation of the measured naphthalene mass per tape strip by Air Force base are shown in Table 1. There was large variation in dermal exposure within and between the Air Force bases. No significant differences in mean exposures (naphthalene mass) between the first, second, and third successive tape strips ($P = 0.96$) were observed in the analysis. For the 1st tape strips the mean and standard deviation were 182 ng and 1430 ng (n = 365), respectively. For the 2nd tape strips, the mean and standard deviation were 198 ng and 1624 ng (n = 222), respectively. For the 3rd tape strips the mean and standard deviation were 224 ng and 1655 ng (n = 165), respectively. This indicates that JP8 is penetrating through the stratum corneum.

When the data was stratified by Air Force base, the highest exposures were observed at Davis-Monthan AFB (Figure 1.). The median exposure was highest at Davis-Monthan AFB, although the other bases had similar median exposures. When stratified by exposure category, the tape strip data displayed a natural descending trend with the exposure categories form HI to LOW (Figure 2.). The HI group had the highest exposures but also the greatest variation in exposure. There was, however, overlap in exposure between the exposure categories.

Discussion/Conclusions

The final results indicate that the tape-stripping methods can be used to measure JP8 contamination on the skin. A wide range of exposures (5 orders of magnitude), even across the individuals considered exposed, were observed. It is important to keep in mind that the exposures captured by the tape-stripping are indicative of what was on the skin at the time of the dermal sampling. This may or may not be representative of the subjects' actual exposure. However, results of the consecutive tape strips show that JP8 is able to penetrate the stratum corneum. Further analysis of the data is aiming to shed light upon the amount actually absorbed into and through the skin and consequently made available for the uptake by the body (total body dose).

Table 1. Naphthalene mass (ng) per tape strip by U.S. Air Force base.

Base	No. of Samples	Mean	Median	SD
DAV	222	543	20	2774
SEY	80	118	4	578
LAN	103	33	0	161
POP	137	34	11	115
LIT	110	28	8	52
HUR	101	59	6	247

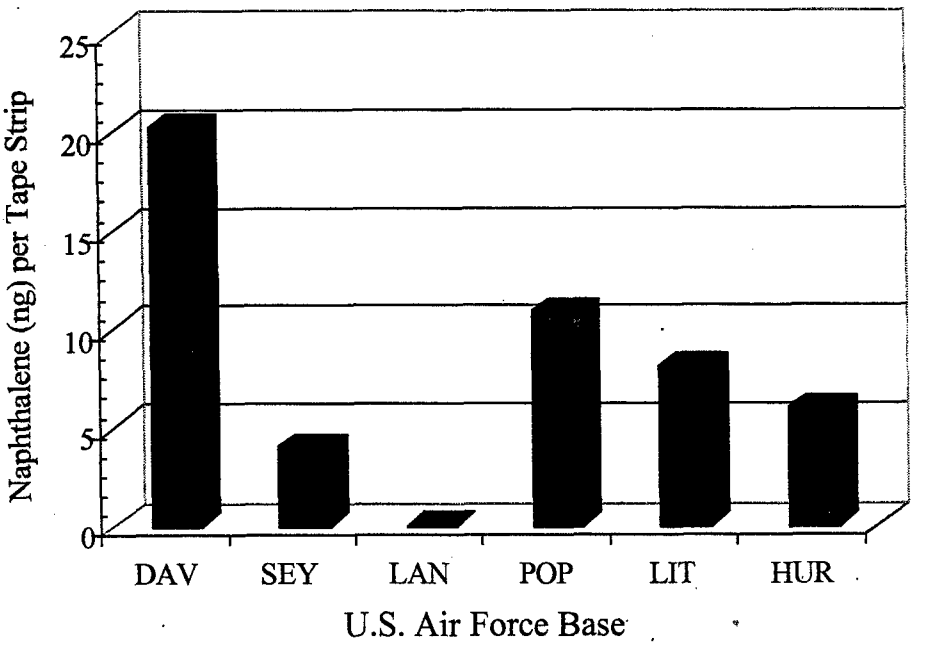


Figure 1. Median naphthalene mass (ng) per tape strip by U.S. Air Force base.

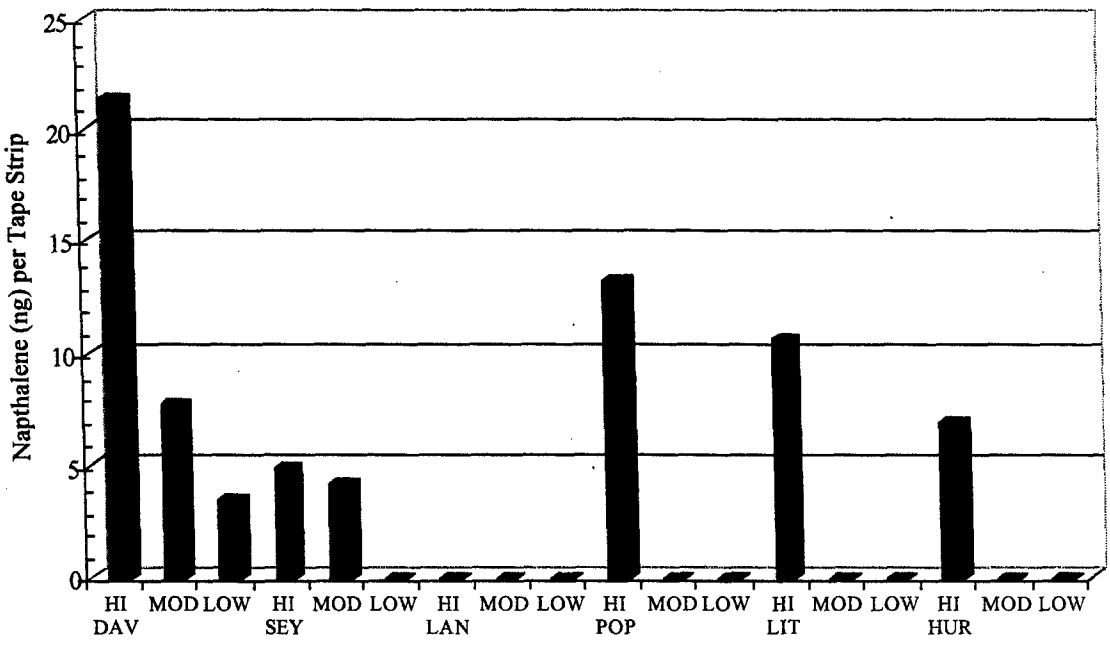


Figure 2. Median naphthalene mass (ng) per tape strip by exposure category.

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Assessment of JP8 in Blood
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Exposure to volatile organic chemicals (VOCs) including jet fuel is a potentially important risk factor to human health from occupational and environmental pollutants. Many of the trace VOCs ubiquitous to everyday experience (e.g., benzene from fuels, chloroform from water, tetrachloroethylene from dry-cleaning, etc.) are recognized for their potential for adversely impacting human health. The standard VOCs technology for assessing exposure and resulting dose has been the direct monitoring of the environment. Typically, environmental samples are collected for analysis and then the potential human impact through inhalation, ingestion, and dermal contact exposure routes extrapolated from the data. The ability to determine direct body burden from exposure to VOCs would allow better understanding of the association between environmental or occupational exposures to VOCs and the subsequent impact on human health. While JP8 body burden measures using breath samples are reasonably well-described, quantifying JP8 in blood and urine specimens remains an area under study. With improved methodologies for measuring JP8 constituents in tissues such as blood, partition coefficients with other body fluids and breath could be determined and physiologically-based pharmokinetic models derived. Because the Risk Assessment of Acute Exposure to Jet Fuel study collected blood, breath and urine samples from subjects during the same time interval, the results of this research may substantially improve the understanding of JP8 associated human health and performance effects.

Methods:

Ten ml blood specimens were obtained from Subjects enrolled in the Risk Assessment of Acute Exposure to Jet Fuel study prior to and following a four-hour work period. The specimens were collected in standard "vacutainer" vials containing an anticoagulent (Heparin). Immediately 6 ml of the blood specimen was transferred to a prepared vial containing 18 ml of a pentane/HCl/internal standard solution. The vial was aggressively shaken or "vortexed" to achieve mixing and then centrifuged to separate the solvent and blood layers. Finally, the clear solvent layer was transferred to a sample vial (on ice). Specimens were shipped to the laboratory in temperature-controlled containers.

At the laboratory, the specimens were reduced in volume by evaporation (Kuderna-Danish) and a small aliquot was then analyzed for JP8 compounds with gas chromatograph mass spectrophotometry (GC-MS).

Status:

Samples were collected from approximately 324 subjects enrolled in the study. The laboratory has currently processed pre- and post-exposure specimens from approximately 164 subjects. Further processing is underway. Problems in determining detection limits hampered analysis of the first 110 specimens and reduced analytical sensitivity. These problems have been resolved and further specimen processing is proceeding as planned. Correlation comparisons with other body burden measures to include breath and urine are yet to be accomplished.

Findings:

Currently, processed blood data cover approximately 50.6% of the enrolled subjects. Specimens were processed for 75 subjects who routinely work with JP8 in the Aircraft Fuel Systems Repair shop (classified for this study as HI exposure) and 86 subjects who normally do not come in contact with fuels or solvents while performed their duties (termed LOW exposure). Additionally, specimens were processed for 3 subjects who work at jobs where exposure to jet fuel occurs intermittently.

Using Pleil's recommended procedure of summing of the nonane, decane, undecane and dodecane concentrations into a single value representing the "JP8 fingerprint" in blood, the values reported range from 0 to 124 ng/ml of blood. Though varying due to differences in fuel stock, the JP8 fingerprint index represents about 15% of the vapor and about 11% of the liquid proportion of the average in the Air Force inventory. The range of exposure values among HI exposed subjects was very large, varying from background levels of less than 1 ng/ml to approximately 124 ng/ml with a mean value of 10.24 and a median value of 6.0. These findings indicate that the "exposed subjects" experience a wide range of exposure and should not be considered as a homogeneous group. Subjects classified as LOW exposure displayed JP8 fingerprint blood values ranging from 0 to 45 ng/ml with a mean value of 2.15 and a median value of near zero. Cursory analysis of the aromatics hydrocarbons in the blood specimens also varies widely, especially from base to base. While some of the variance in JP8 constituents in blood may be due to subject-level differences in on-the-job exposures, variations in fuel components may also play a role.

Conclusions:

While the levels of JP8 constituents in blood may ultimately serve as the "gold standard" for measure of JP8 body burden, the wide variability seen in the specimens analyzed for this study bring the value of this type of analysis into question. Although comparisons between JP8 exposure blood values and other markers of JP8 exposure (breath, urine, and ambient air monitoring) have not yet been addressed, cursory comparisons show little or no correlation between blood and breath measures. These finding indicate that further information may be needed to fully understand the implications of specific types of JP8 exposure. Nevertheless, the blood data are useful indicators for aggregate JP8 exposure because they represent exposures from multiple routes, including dermal and inhalation. The composite JP8 fingerprint (the sum of nonane, decane, undecane, and dodecane) is a good, stable, independent variable for exposure, and should be used to rank subjects both internally at a given base and across bases, because this parameter tends to smooth compositional variability of different fuels and days. The post-exposure data range of nominally-exposed subjects overlaps with that of the nominally-unexposed subjects suggesting that simple division between subject groups is a weak independent variable for correlation to putative health effects. Given the enhanced sensitivity of the blood analysis process, the study may be able to report less variability among subsequently analyzed samples.

If this technology proves effective, the combination of blood and breath data will unambiguously describe the body burden and exposure of a single individual. Additionally, once the relationships are established, the non-invasive breath measurement will serve as the blood measurement surrogate and thus extend the ability to rapidly to a large group of subjects.

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Direct measurement of total body burden of JP-8 jet fuel (Breath)

Risk Assessment of Acute Exposure to Jet Fuel

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U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Introduction:

Human exposure assessment to volatile organic chemicals (VOCs) is an important subset of the overall requirements for characterizing risk from environmental pollutants. The standard VOCs technology for assessing exposure and resulting dose has been the direct monitoring of the environment (air, water, soil, etc.). Over the past few years, scientists have developed sampling and analysis technology to directly assess exposure through measuring the VOCs content of venous blood samples and individual alveolar exhaled breaths. Such biological monitoring is an unambiguous measure of exposure to exogenous chemicals because it reflects all exposure routes and is specific to each individual subject. Breath is preferable to blood as a biological medium for VOCs exposure because collection is noninvasive, is relatively simple, and does not generate potentially infectious waste. Breath directly reflects the blood VOCs concentration; this is the basis of the classic police "breathalizer" test for ethanol inebriation where a breath measurement is interpreted as a "blood alcohol" level. Using breath samples, we no longer have to extrapolate from ambient data to calculate potential exposure and we can avoid the complications of blood sampling and analysis.

Methods:

Specifically, breath samples were collected in one-liter volume evacuated canisters equipped with small Teflon™ breathing tubes. The subject established a regular breathing pattern and then self-administered a breath collection by closing his lips on the breathing tube as the canister valve was opened during an exhalation. Once the canister vacuum was filled with breath, the valve was closed. The breath samples were then transported to the laboratory and analyzed via trace-level gas chromatography - mass spectrometry methods similar to those for ambient air. The details and some applications of this method have been published in the peer-reviewed literature (1,2). From previous knowledge of the composition of the jet fuel, the analytical system was externally calibrated to allow quantitative determination of key constituents of fuel expected to be found in breath. Specifically, these were single ring aromatic hydrocarbons (benzene, toluene, ethylbenzene, m,p-xylene, o-xylene, 4-ethyltoluene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene and styrene) and C_4 to C_{12} n-alkanes. All samples were collected before and after the nominal exposure period. Initially, subjects were randomly chosen among nominally exposed and unexposed cohorts; later in the study, it was determined that the primary focus was to be on the exposed group due to resource constraints. Adequate duplicate samples were collected

to allow good precision statistics to be calculated. All samples were additionally assayed for carbon dioxide and oxygen content to assure their validity as breath samples. The specific JP-8 jet fuel exposure methods using breath biomarkers and prior survey data have been published (3).

Status:

All samples have been cataloged, analyzed, validated, and results have been entered into Lotus 123 spreadsheets. Final data in the form of ppbv (parts per billion by volume) concentration for all assayed compounds (see list above) for all sampled subjects were submitted to the Air Force and to TTU in January, 2001. Of these, 114 subjects were nominally exposed and 6 were nominally unexposed. Additionally, we submitted data for 20 subjects from the initial practice study at Dyess AFB. Final consolidated results were presented at the International Jet Fuel Conference in San Antonio, TX (4). Data tables of relative exposure rankings, aromatic composites and fingerprint composites were made available to other researchers at the meeting.

Findings/Discussion:

The EPA breath data cover about 70% of the nominally exposed subjects. To facilitate the data analysis and relative exposure comparisons, a single value of the sum of the nonane, decane, undecane, and dodecane concentrations in breath can be used as the "JP-8 fingerprint". Though varying due to differences in fuel stock, this JP-8 fingerprint number represents about 15% of the fuel vapor, and about 11 to 17% of the liquid. The range of exposure among nominally exposed subjects is very large, varying from background levels of less than 1 ppbv up to about 1250 ppbv JP-8 fingerprint value. As such, the group of "exposed subjects" contain the full range of exposure and cannot be used as a homogeneous group. Figure 1 is a bar chart demonstrating this range of exposure of nominally "exposed" subjects. Cursory inspection of the aromatics data also indicate wide variability, especially from base to base. For instance, at Little Rock AFB, the sum of BTEX (benzene, toluene, ethylbenzene, m,p,o-xylenes) is about 14% of the JP-8 fingerprint value on average for post-exposure subjects, whereas at Pope AFB the sum of BTEX is about 50% of the JP-8 fingerprint. Some of this may be attributable to subjects' behavioral differences (i.e. smoking), however, these findings imply that fuel components may be much more variable than originally expected. Exposures vary greatly among bases as indicated in Figure 2 where the mean and standard error of the mean are indicated for ranked quartiles for each base. Note that the 1st quartile at Langley is lower than the 3rd quartile of Little Rock, Pope, and Hurlburt suggesting that exposure categories should be constructed from overall, aggregate data lists.

All exposure concentration data have now been reduced to tables in a series of spreadsheets. In addition to tables of speciated data (per subject, per base, per compound and for both pre and post-exposure), the overall population of the 120 subject tested has been ranked from highest to lowest body burden as measured in exhaled breath fingerprint. Blank values and estimates of error bars for various JP-8 constituent measurements are provided. The tabulated data and the ranking information have been provided to interested health effects researchers, and are available upon request to any of study Principal Investigators.

Conclusions:

The breath data are useful indicators for aggregate exposure because they represent both dermal and inhalation exposure routes. The composite JP-8 fingerprint (the sum of nonane, decane, undecane, and dodecane) is a good, stable independent variable for exposure and should be used to rank subjects both internally at a given base and across all bases because this parameter tends to smooth compositional variability of different fuels and days. The post exposure data range of nominally exposed subjects overlaps with that of the nominally unexposed subjects and the pre-exposure samples suggesting that simple divisions among exposed, moderately exposed, and unexposed subject groups is a weak independent variable for correlation to putative health effects. The small (10%) group of subjects with the highest post-exposure breath concentrations should be further investigated to see if there are work practices changes that could reduce their exposure to the mean values of their peers.

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4. Pleil, J.D. and M. Colon, 2001. "Breath Measurement of Total Body Burden of JP-8 Jet Fuel for Epidemiological Study", International Conference on Jet Fuel Exposure, San Antonio, TX, August 2001.

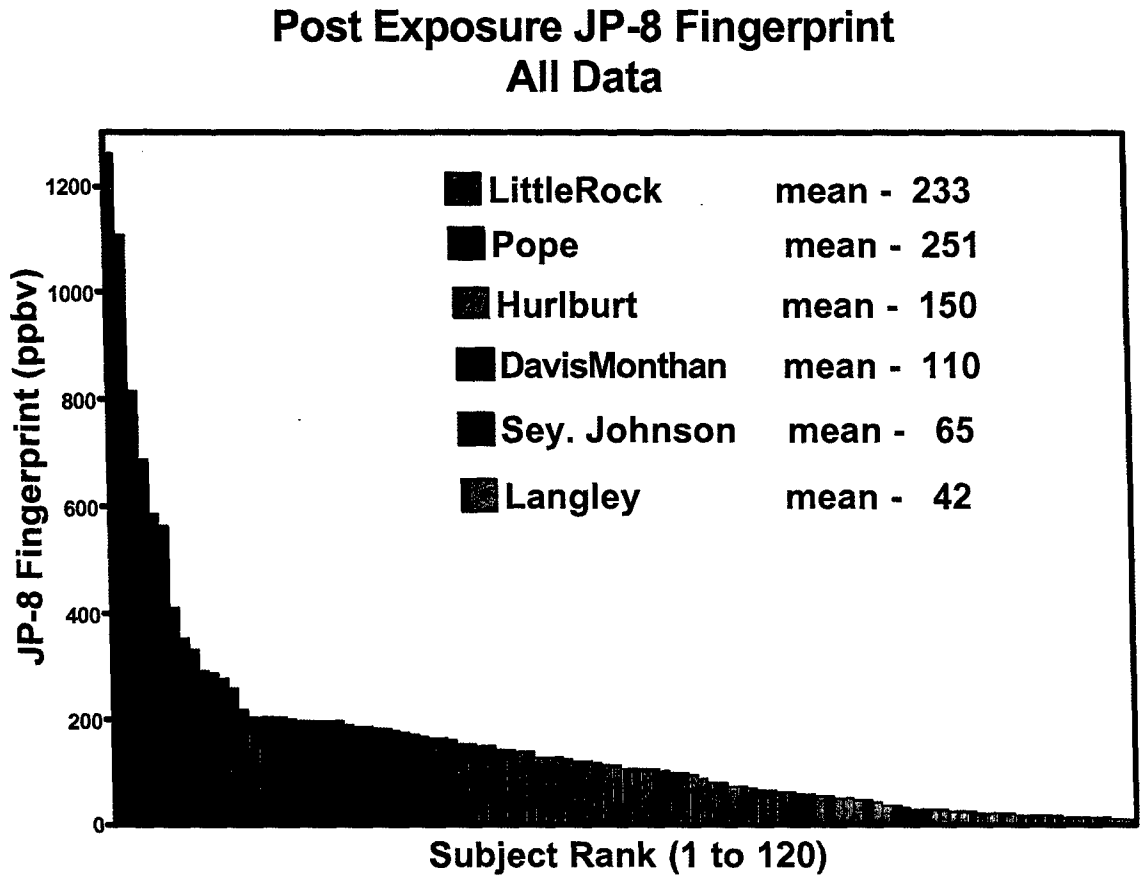

Figure 1. All subjects ranked highest to lowest by "JP-8 Fingerprint" in their post exposure breath

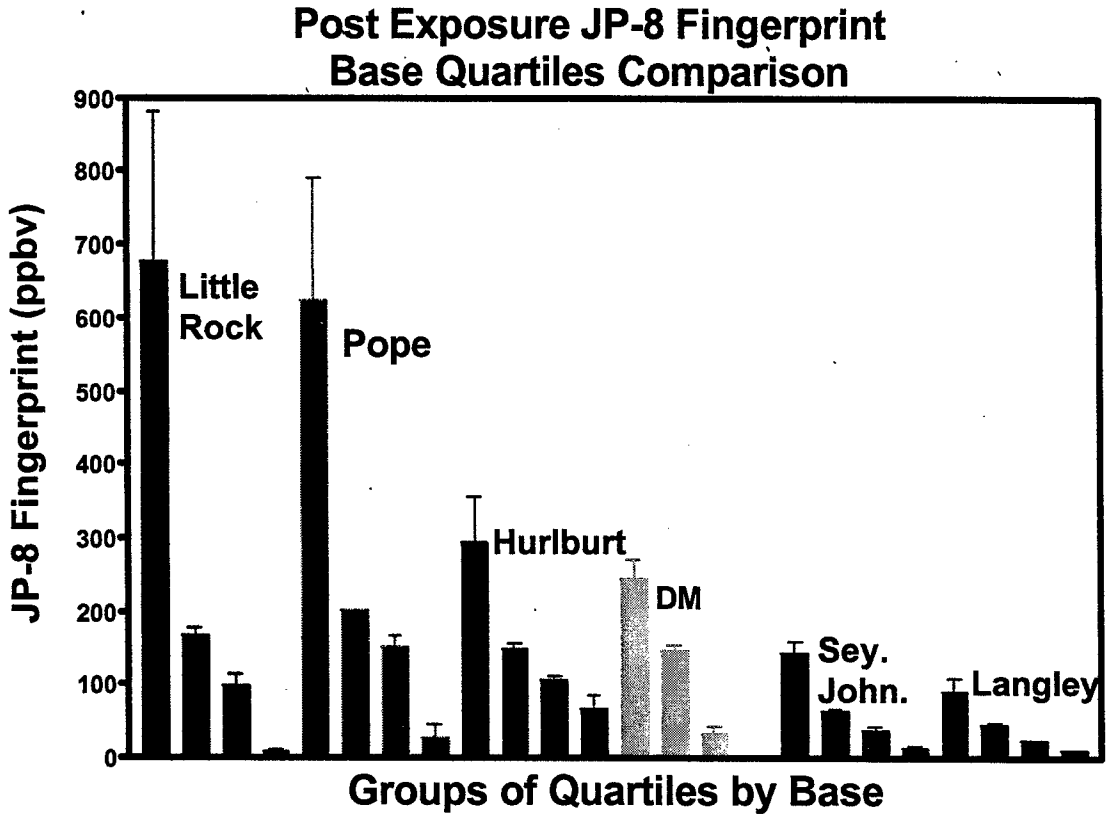

Figure 2. Base comparison by JP-8 fingerprint quartiles

Measurement of Benzene and Naphthalene in Air and Breath in the U.S. Air Force as an Indicator of JP8 Exposure

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ABSTRACT

We measured benzene and naphthalene in air and breath among U.S. Air Force personnel placed into three exposure categories based on job codes. Median benzene concentrations in air were 3.1, 7.4, and 252 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <1.0 – 6629 $\mu g/m^3$. Median naphthalene concentrations in air were 1.9, 10.3 and 485 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <1.0 – 3911 ug/m^3. The median post-exposure concentrations of benzene in breath among all participants were 4.6, 9.0, and 11.4 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <1.5 – 153 $\mu g/m^3$. The median post-exposure concentrations of naphthalene in breath among all participants were 0.73, 0.93, and 1.83 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <0.5 – 15.8 $\mu g/m^3$. Relationships between air and breath were weak for the low and moderate exposure categories. Significant linear correlation was observed in the high exposure category, with correlation coefficients of 0.59 ($n = 114$, $p < 0.0001$) for benzene and 0.62 ($n = 109$, $p < 0.0001$) for naphthalene. Multiple linear regression analysis using data from fuel tank repair workers found primary role in the maintenance work, storage location of fire suppression foam, ambient temperature, cross-ventilation, and purpose of the work to be important factors in explaining variability in exposure. Final models for benzene and naphthalene in breath had concentration in air and skin irritation in common. The model for benzene in breath also included respirator use, physical exertion, and cigarette smoking, and the model for naphthalene also included temperature, tank purge method, and orientation of tank entry. Models explained the variability in naphthalene in breath better than the variability in benzene in breath.

INTRODUCTION

JP8 is used throughout the U.S. Air Force as fuel for aircraft as well as for military vehicles and auxiliary ground equipment. Even those workers whose duties do not involve direct contact with jet fuel have incidental exposure to fuel vapors (Pleil et al., 2000). Among those with direct contact, there has been increasing concern about potential health effects. Evaluating the possibly deleterious health effects arising from JP8 exposure necessitates quantifying the levels to which individuals are exposed.

Concentrations of the unchanged parent compound in exhaled air reflect the actual body burden derived from all routes and sources of exposure and account for individual differences in physiology and work practices (Lowery, 1986). Few previous studies of jet fuel exposure (LeMasters et al., 1999; Pleil et al., 2000) have included biological monitoring in a comprehensive manner. In this study, biological and external monitoring were used together to measure benzene and naphthalene in both the breathing zone during the workshift and in exhaled breath before and after work. Benzene, a known human carcinogen, is a minor constituent of JP8, found at a concentration of about 0.01% or below (IARC, 1989; Puhala et al., 1997; Carlton and Smith, 2000). Naphthalene, one of the major aromatic constituents, is present at about 0.26% (McDougal et al., 2000) and has previously been identified as a marker of jet fuel in studies of dermal exposure (Riviere et al., 1999; Kanikkannan et al., 2001a; Kanikkannan et al., 2001b). Measurements were supplemented with information related to the particular tasks, procedures, and the workplace environment obtained via questionnaires. Multiple regression procedures were used to evaluate factors important in determining exposure and in explaining the variability in the relationship between concentration in breath and in air for both benzene and naphthalene.

METHODS

Air and Breath Monitors

The passive air monitors were custom-fabricated aluminum tubes (90 mm x 6.3-mm o.d. x 5.0-mm i.d.) containing 0.1 g of 20/35 mesh Tenax TA (SKC Inc., Eighty Four, PA) with an open diffusion channel of 1.5 cm x 5.0-mm i.d. This sampler is similar to a commercial device that has undergone extensive environmental testing (Brown, 1999). The breath samplers were custom-fabricated glass bulbs (75-mL volume, 13-cm length) sealed with threaded, plastic caps (Chemglass, Vineland, NJ). The subject was instructed to remove the end caps and completely exhale through the bulb following a normal inhalation. Since the bulb volume was small compared to the vital capacity, only end-exhaled air was collected (based upon measurements of CO_2, Egeghy et al., 2000). Previous recovery experiments confirmed minimal losses during storage for at least 4 weeks (Egeghy et al., 2000). Upon receipt at the laboratory, breath samplers were checked for loose or deformed caps and for the presence of condensed water vapor as an indicator of sample integrity.

Analysis of Monitors

Monitors were shielded from light and stored at room temperature for less than 3 weeks before analysis. Directly before GC analysis, breath samples were passively transferred from the bulbs to sorbent tubes of the type described above for air monitoring. One cap was removed from the bulb, whereupon a sorbent tube was quickly placed inside and the cap replaced. After 24 h, the sorbent tube was removed and sealed prior to analysis.

All samples were desorbed with a Perkin Elmer ATD 400 automatic thermal desorption system (Periago et al., 1993) for 2 min at 225 °C to transfer analytes onto a Tenax-packed, cryogen-free focusing cold trap maintained at -30 °C. The cold trap was then rapidly heated to 225 °C and held at that temperature for 0.1 min to transfer the contents to the analytical column via a fused silica transfer line, maintained at 200 °C. Benzene and naphthalene were measured with a Hewlett Packard 6890 Series II gas chromatograph (Hewlett Packard Corp., Palo Alto, CA) equipped with HNU PI-52-02A photoionization detector (PID) with a 9.5 eV lamp (HNU Systems, Inc., Newton, MA). Separation was achieved with a megabore DB-1 column of 60-m x

0.53-mm i.d. dimethylpolysiloxane (1.5 μm film thickness) (J&W Scientific, Folsom, CA). Ultra high purity helium was used as the carrier gas at a flow of 8 ml/min. The oven temperature was held at 40 °C for 5 min, increased to 75 °C at 10 °C/min, then increased at 5.5 °C/min to 175 °C, and then increased at 50 °C/min to 260 °C where it was held for 6 min. Chromatograms were manually integrated using Hewlett Packard GC ChemStation software. Benzene and naphthalene were identified by the retention times of 6.05 min and 21.95 min, respectively.

Samples were quantified against external benzene standards prepared in Tedlar bags (SKC Inc., Eighty Four, PA) by serial dilution of liquid benzene (99.9%, Fisher Scientific, Pittsburgh, PA) with zero-grade air and against external naphthalene standards prepared by spiking sorbent tubes with 2 μl of known concentrations of naphthalene (99.9%, Fisher Scientific, Pittsburgh, PA) in hexane prepared by serial dilution. Calibration curves were determined by linear least-squares regression. The limits of quantitation (LOQs) were 1.5 and 0.5 $\mu g/m^3$ for benzene and naphthalene, respectively, in the breath monitors and 1.0 $\mu g/m^3$ for both compounds on the air monitors (4-h sampling duration). LOQ was based on three times the average residual benzene peak from analysis of unexposed air samplers.

Statistical Analysis

Not all subjects had a complete set of measurements of benzene and naphthalene in air and breath. Some measurements did not satisfy requirements for quality control because of loose caps and/or lack of condensed water and were excluded from statistical analysis while others were lost to equipment malfunction. Observations below the LOQ were assigned values of 2/3 LOQ prior to statistical analysis (Hornung and Reed, 1990).

All statistical analyses were performed using SAS Statistical Software (SAS Institute, Cary, NC). Median benzene and naphthalene concentrations were compared among and within exposure groups using rank-sum tests for medians available with the NPAR1WAY procedure of SAS. All other analyses employed (natural) logarithmic transformation to remove heteroscedasticity and better satisfy normality assumptions.

Multiple linear regression analysis was performed using the GLM procedure of SAS to separately investigate determinants of benzene and determinants of naphthalene in air and in breath. Manual forward stepwise selection was used to build models using measured concentrations and information from various questionnaires. As suggested by Hosmer and Lemeshow (1989), the outcome variables were first regressed on each independent variable separately to eliminate unlikely predictors. In the subsequent forward selection process, only those variables with a significance level of $p < 0.10$ were retained in the model.

RESULTS

Benzene and Naphthalene in Air and Breath

Measurements of benzene in air and breath are grouped by exposure category and summarized in Table 1. The median benzene concentrations in air were 3.1, 7.4, and 252 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <1.0 – 6629 $\mu g/m^3$. The median pre-exposure breath concentrations of benzene were 4.7, 5.8, and 4.6 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <1.5 – 104.4 $\mu g/m^3$. The median post-exposure concentrations of benzene in breath among all subjects were 4.6, 9.0, and 11.4 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <1.5 – 153 $\mu g/m^3$.

Measurements of naphthalene in air and breath are summarized in Table 2. The median naphthalene concentrations in air were 1.9, 10.3 and 485 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <1.0 – 3911 ug/m^3. The median pre-exposure concentrations of naphthalene in breath were <0.5, 0.58, and <0.5 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <0.5 – 36.3 $\mu g/m^3$. The median post-exposure concentrations of naphthalene in breath among all subjects were 0.73, 0.93, and 1.83 $\mu g/m^3$ in the low, moderate, and high exposure categories, respectively, with an overall range of <0.5 – 15.8 $\mu g/m^3$.

Relationship Between Air and Breath Levels

The scatter plots in Figure 1 illustrate the relationship between concentrations in air and breath for all subjects, with benzene (Figure 1A) and naphthalene (Figure 1B) plotted separately. Overall, significant linear correlations were observed between the logged exposures and logged breath concentrations for both benzene ($r = 0.55$, $n = 290$, $p < 0.0001$) and naphthalene ($r = 0.67$, $n = 279$, $p < 0.0001$). The scatter plots in Figure 2 illustrate the relationship between concentrations in air and breath for subjects grouped by exposure category, again with benzene and naphthalene plotted separately. For benzene, the Pearson correlation coefficients were 0.18 ($n = 130$, $p = 0.04$), 0.24 ($n = 35$, $p = 0.16$), and 0.59 ($n = 114$, $p < 0.0001$) in the low, moderate, and high exposure categories, respectively, while for naphthalene the coefficients were 0.27 ($n = 126$, $p = 0.002$), 0.44 ($n = 35$, $p = 0.0085$), and 0.62 ($n = 109$, $p < 0.0001$) for the same categories. The relationship among the four types of measurements from all subjects is displayed as Spearman correlation coefficients in Table 4; all correlations are significant at $p < 0.0001$.

Model Fitting

Final models for benzene and naphthalene in air and in post-exposure breath are summarized in Tables 5, 6, 7, and 8. The final model for benzene in air (Table 5), with a coefficient of determination (r^2) of 0.64, included primary role (PRIMARY; 1 = entrant, 2 = attendant, 3 = runner/ fireguard/other), purpose of work (PURPOSE; 1 = inspect, 2 = find leak, 3 = repair), foam storage location (STOREFOAM; 1 = wing, 2 = floor, 3 = enclosed container), temperature (TEMPERATURE; 2 = 50 – 70 °F, 3 = 70 – 90 °F, 4 = >90 °F), total length of breaks (BREAKLONG; h), and cross ventilation (CROSSVENT; 1 = yes, 2 = no).

The final model for naphthalene in air (Table 6) was very similar to the model for benzene; the model included PRIMARY, PURPOSE, STOREFOAM, and CROSSVENT. Additionally, the model included general distance from tank during fuel work (TANKDIST; 1 = inside, 2 = less than 10 ft, 3 = more than 10 ft). The coefficient of determination for the final model (r^2) was 0.64.

As summarized in Table 7, the final model for the post-exposure concentration of benzene in breath (BZ_{post}) had an r^2 of 0.62 and included seven main effects, namely: benzene in air (BZ_{air}; $\mu g/m^3$), pre-exposure breath concentration (BZ_{pre}; $\mu g/m^3$), smoking activity (SMOKED; 1 = yes, 2 = no), physical job stress on day of sampling (PEXERT; ordinal scale based on exertion measures), self-reported skin irritation from contact with liquid jet fuel (IRRITATION, 1 = yes, 2 = no), respirator use (RESPIRATOR; 1 = yes, 2 = no), and time between end-of-exposure and post-exposure breath samples (TRAVELTIME; min),. No interaction terms were significant at $p < 0.10$.

The final model for the post-exposure breath concentration of naphthalene (NAP_{post}) (Table 8, $r^2 = 0.66$) included five main effects, namely: naphthalene in air (NAP_{air}; $\mu g/m^3$), orientation of fuel tank entry (HOWENTER; 1 = did not enter, 2 = from top, 3 = from bottom), tank fuel vapor purge method (PURGE; 1 = supplied air, 2 = exhaust, 3 = combination), IRRITATION, and TEMPERATURE.

DISCUSSION

Benzene and Naphthalene in Air and Breath

Two separate populations of workers, "JP8 exposed" and "unexposed," were recruited but each subject was eventually assigned to one of three exposure categories (low, moderate, or high) based on his or her primary career field (AFSC, or Air Force Specialty Code). Fuel system repair workers, considered by Air Force industrial hygienists to have the highest exposure to jet fuel because they routinely enter fuel tanks (Carlton and Smith, 2000), were heavily recruited and assigned to the "high" category. Subjects having regular physical contact with jet fuel through work activities such as fuel handling, distribution, recovery, and testing were assigned to the "moderate" category. Subjects whose jobs did not involve routine contact with fuels during performance of their duties were assigned to the "low" category.

The median concentration of naphthalene in air measured in the breathing zone of subjects in the low category (1.9 $\mu g/m^3$) was about four-fold higher than the 0.3 – 0.7 $\mu g/m^3$ commonly found in ambient air (CARB, 1998), suggesting incidental exposure to jet fuel and jet fuel exhaust from JP8-fueled aircraft and ground support equipment (Childers et al., 2000; Pleil et al., 2000). The median benzene concentration (3.1 $\mu g/m^3$), however, was below the global average outdoor concentration of 6 $\mu g/m^3$ (Wallace, 1996). The median concentrations of both naphthalene (10.4 $\mu g/m^3$) and benzene (7.45 $\mu g/m^3$) in air among subjects in the moderate category were significantly higher than those among subjects in the low category ($p < 0.0001$, Mann-Whitney U test of medians, not corrected for multiple comparisons).

The median exposures to naphthalene (447 $\mu g/m^3$) and benzene (242 $\mu g/m^3$) in air among subjects in the high category were about 30 – 40 fold higher than in the moderate category. Naphthalene levels have not been previously reported among jet fuel exposed populations, but the maximum benzene exposure we measured (6,629 $\mu g/m^3$) was about twice as high as the maximum 8-hr TWA benzene exposure of 3,300 $\mu g/m^3$ reported by Carlton and Smith (2000). However, Carlton and Smith also reported benzene concentrations as high as 49,100 $\mu g/m^3$ and Pleil et al. (2000) reported a mean benzene concentration (n = 16) of 2,987 ppb (9,540 $\mu g/m^3$) inside fuel tanks. Hence, an exposure of 6,629 $\mu g/m^3$ is plausible for a subject who spends an extended period of time inside the tank. It should be noted, however, that these data do not represent valid estimates of long-term exposure among fuels workers since activities were coordinated to guarantee maintenance procedures requiring tank entry on each sampling day.

Exposure to naphthalene in air (Table 2) was significantly different among the three categories ($p < 0.0001$, Kruskal-Wallis test) with a trend consistent with the *a priori* expectation. The three distributions of the log-transformed values are plotted in Figure 4. The low and high categories are clearly separate populations, but the moderate category overlaps both. Exposure to benzene in air (Table 1) was also significantly different among the three categories ($p < 0.0001$, Kruskal-Wallis test) with a similar trend, but with more overlap in the distributions (Figure 5).

In the low category, the median benzene concentration in breath was about the same before and after work activities (4.7 and 4.6 $\mu g/m^3$, respectively), but the median naphthalene concentration increased slightly from < 0.5 $\mu g/m^3$ (LOD) to 0.73 $\mu g/m^3$. In the moderate category, both the median benzene and the median naphthalene concentrations in breath were roughly 1.5 times higher following exposure. In the high category the median benzene and naphthalene concentrations in breath were 2.5 and 3.2 times higher, respectively, following exposure. Despite higher concentrations of naphthalene than benzene in air, median naphthalene levels in post-exposure breath were much lower than median benzene levels. Because naphthalene has a lower vapor pressure and much higher blood-to-air partition coefficient (NTP, 2000), it is readily absorbed into the blood but not excreted through the lungs as efficiently as benzene.

In nonparametric tests of medians without correction for multiple comparisons (Table 3), the difference in naphthalene concentrations in breath between the low and moderate groups was only marginally significant ($p = 0.0465$, one-sided Mann-Whitney U). The difference in median naphthalene concentrations between the moderate and high groups was highly significant ($p < 0.0001$) despite the confounding effect of respirators (only 2% of subjects in the moderate group wore respirators whereas 96% of the subjects in the high group wore them for some portion of the exposure period). Comparison of the distributions of naphthalene concentrations among the three exposure categories shows much less overlap in air (Figure 4) than in breath (Figure 6). indicating that the differences in body burden are much smaller that the external exposure measurements would suggest. Plots of the distributions of benzene in air and breath by exposure category (Figures 5 and 7, respectively) show a similar pattern but with less differentiation between the moderate and high categories.

Relationship Between Air and Breath Levels

The relationship between concentrations in air and in breath was weak for both benzene and naphthalene among subjects in the low and moderate categories, with confounding by cigarette smoking evident for benzene but not for naphthalene (Figure 2). Although the relationship was much stronger in the high category, substantial variability remained. Similar relationships have been reported in field studies (Perbellini et al.,1988; Egeghy et al., 2000) and controlled human exposures (Pierce et al., 1996).

Model Fitting

Four separate multiple regression analyses were performed to analyze determinants of benzene and naphthalene in air and breath among aircraft fuel system workers. The dependent variables were both benzene and naphthalene in air and in post-exposure breath.

The final models for benzene and naphthalene in air (Tables 5 and 6) were very similar to each other. Both models included primary role of the worker, foam storage location, cross-ventilation, and the purpose of activities. Those whose primary role was "entrant" were associated with the highest exposures, agreeing with previous studies reporting much higher benzene concentrations inside the tank (Carlton and Smith, 2000; Yeung et al., 1997; Pleil et al, 2000). Cross ventilation, often created inside hangars by opening both the main and the auxiliary doors, was associated with lower exposures. Storing foam on the wing, which keeps it out of the way and requires less handling than carrying it to the floor, was also associated with lower exposures. Finding a leak was associated with lower exposures than either performing an inspection or a repair. Higher temperatures, which may limit the amount of time that a worker

spends inside the tank (where concentrations are highest), resulted in lower exposures. For benzene only (Table 5), longer breaks were also associated with lower exposures. For naphthalene only (Table 6), distance from tank during fuel work was a significant predictor, with exposure highest for those who reported their general distance from the tank as "inside" and lowest for those who reported "more than 10 ft."

The final model for benzene in post-exposure breath (Table 7) included the concentration of benzene in air and in pre-exposure breath. Respirator use was associated with lower benzene concentrations in breath; respirators were always worn during tank entry and often worn when opening the fuel tank and removing foam. Concentrations in breath were higher among those who reported skin irritation (irritation, cracking, or burning) resulting from contact with fuel. Physical exertion on the day of sampling, as determined by exertion measures by questionnaire, was found to have a significant positive association with concentration in breath; physical exertion increases both breathing rate and cardiac output, both of which are believed to increase inhalation absorption (Gardner and Kirkpatrick, 1998). The time that it took workers to clean up, travel to the testing center, and be seated for testing ("TRAVELTIME") had a significant negative effect on benzene concentration in breath. As expected, cigarette smoking increased benzene concentrations.

In the final model for naphthalene in post-exposure breath (Table 8) cigarette smoking did not have a significant effect. Naphthalene is present in tobacco smoke at much lower levels than benzene (1.2 – 3 μg naphthalene vs. ~55 μg benzene per cigarette) (ASTDR, 1995; Wallace, 1996; Yang et al., 1999). Tank entry from the bottom of the aircraft, which generally resulted in greater contact with fuel, was associated with higher breath levels than either tank entry from the top or no entry. The model also included two other variables that suggest dermal exposure may have had an important role in determining naphthalene in breath, namely: skin irritation and ambient temperature. Higher naphthalene level in breath was associated with self-report of irritation, cracking, or burning of the skin and with higher ambient temperatures. Experiments on rat and pig skin have found that naphthalene penetrated skin better than the aliphatic components of JP8 and that JP8 caused skin irritation and that JP8 disrupted the barrier function of skin (McDougal et al, 2000 Kanikkannan et al., 2001). Regarding temperature, dermal blood flow is known to increase with ambient temperature (Terreros, 1999) facilitating greater dermal absorption. The method used for purging the tank of fuel vapor before entry explained some of the variability in breath concentration; blow or combination purge reduced the concentration of naphthalene in breath, possibly because workers often would take advantage of the supply air vent to cool their faces and to breath fresh air instead of contaminated air. The model for naphthalene in breath explained more variability than the one for benzene (R^2 = 0.66 and 0.62, respectively)

CONCLUSIONS

Concentrations of naphthalene were higher than concentrations of benzene in air, but lower in breath, demonstrating that the lower volatility and higher expected blood:air partition coefficient of naphthalene reduced that amount excreted in breath relative to benzene. The relationship between concentrations in air and in breath were weak for subjects in the low and moderate categories, and although the relationship was much stronger in the high category substantial variability remained. The relationship was stronger for naphthalene than for benzene. Our multiple linear regression models incorporating concentration in air with factors related to

the individual and to the work environment were better able to explain the variability in naphthalene ($R^2 = 0.66$) than in benzene ($R^2 = 0.62$) in post-exposure breath.

For both benzene and naphthalene, significant differences were found between concentrations in air among the three exposure categories; however, the categories, which were based primarily on job titles (Air Force Specialty Codes), were far less distinct for concentrations in breath. Since health effects are thought to be more closely related to body burden than to external concentrations, and since concentration in breath is believed to closely represent body burden, use of the current classification scheme in evaluating health effects may introduce significant misclassification error.

TABLES

Table 1. Summary of levels of benzene in environmental air and breath.

Variable	Exposure Category	n	# < LOD	Percent < LOD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum	Mean
Benzene in Air	High	114	0	0%	6.1	75.7	251.8	863.5	6629.2	931.4
Benzene in Air	Moderate	38	0	0%	1.4	4.0	7.4	32.5	1851.3	137.5
Benzene in Air	Low	140	1	0.7%	< 1.0	2.2	3.1	4.8	61.3	4.5
Benzene in Breath, pre-exposure	High	111	19	17.1%	< 1.5	2.6	4.6	8.2	30.4	6.1
Benzene in Breath, pre-exposure	Moderate	44	3	6.8%	< 1.5	3.7	5.8	9.3	25.2	7.1
Benzene in Breath, pre-exposure	Low	151	23	15.2%	< 1.5	2.9	4.7	7.4	104.4	7.4
Benzene in Breath, post-exposure	High	114	2	1.8%	< 1.5	6.5	11.4	24.4	152.9	20.4
Benzene in Breath, post-exposure	Moderate	41	3	7.3%	< 1.5	3.6	9.0	20.3	58.1	13.3
Benzene in Breath, post-exposure	Low	143	11	7.7%	< 1.5	3.1	4.6	7.8	49.9	7.7

Table 2. Summary of levels of naphthalene in environmental air and breath.

Variable	Exposure Category	n	# < LOD	Percent < LOD	Minimum	Lower Quartile	Median	Upper Quartile	Maximum	Mean
Naphthalene in Air	High	113	0	0%	12.8	178.7	485.3	867.5	3910.8	659.8
Naphthalene in Air	Moderate	38	3	7.9%	< 1.0	2.2	10.3	29.8	932.1	61.9
Naphthalene in Air	Low	139	30	21.6%	< 1.0	1.1	1.9	3.2	16.9	2.7
Naphthalene in Breath, pre-exposure	High	112	77	68.8%	< 0.5	< 0.5	< 0.5	0.6	6.1	0.6
Naphthalene in Breath, pre-exposure	Moderate	43	20	46.5%	< 0.5	< 0.5	0.6	0.8	16.1	1.0
Naphthalene in Breath, pre-exposure	Low	149	89	59.7%	< 0.5	< 0.5	< 0.5	0.7	36.3	0.9
Naphthalene in Breath, post -exposure	High	111	7	6.3%	< 0.5	0.9	1.8	4.0	15.8	2.9
Naphthalene in Breath, post -exposure	Moderate	40	12	30.0%	< 0.5	< 0.5	0.9	1.9	13.0	1.5
Naphthalene in Breath, post -exposure	Low	143	51	35.7%	< 0.5	< 0.5	0.73	1.0	6.9	0.85

Table 3. Comparison (p-values) of median concentrations in breath.

	Concentration in Air		Concentration in Breath	
Comparison	Naphthalene	Benzene	Naphthalene	Benzene
low vs. moderate	<0.0001	<0.0001	0.0465	0.0075
moderate vs. high	<0.0001	<0.0001	<0.0001	0.0192

Levels of significance from Mann-Whitney U test of medians, not corrected for multiple comparisons.

Table 4. Spearman correlation coefficients between measures, all subjects.

	BZ_{air}	NAP_{air}	BZ_{post}	NAP_{post}
Benzene in Air [BZ_{air}]	1	0.842	0.491	0.502
Naphthalene in Air [NAP_{air}]		1	0.471	0.622
Benzene in Breath, post-exposure [BZ_{post}]			1	0.520
Naphthalene in Breath, post-exposure [NAP_{post}]				1

All correlations are significant at $p < 0.0001$.

Table 5. Final model for benzene in air.

Effect	Estimate	Std Err	p-Value
Intercept	3.853	0.797	
BREAKLONG (hr)	-0.888	0.348	0.0122
CROSSVENT (yes)	-0.506	0.227	0.0283
PRIMARY	–	–	<.0001
entrant	2.758	0.466	<.0001
attendant	1.568	0.501	0.0023
other	0	-	-
TEMPERATURE	–	–	0.0130
50 – 70 °F	1.181	0.487	0.0171
70 – 90 °F	0.724	0.267	0.0081
> 90 °F	0	-	-
PURPOSE	–	–	<.0001
inspect	-1.229	0.340	0.0005
find leak	-3.108	0.568	<.0001
repair	-1.081	0.344	0.0022
other	0	-	-
STOREFOAM	–	–	<.0001
wing	-0.235	0.581	0.6862
floor	1.202	0.572	0.0382
enclosed	0	-	-

Table 6. Final model for naphthalene in air.

Effect	Estimate	Std Err	p-Value
Intercept	5.158	0.540	<.0001
CROSSVENT (yes)			
PRIMARY	–	–	0.0006
entrant	0.808	0.348	0.0223
attendant	-0.073	0.339	0.8307
other	0	-	-
TANKDIST	–	–	0.0357
inside	0.981	0.408	0.0180
< 10 ft	0.621	0.385	0.1102
> 10 ft	0	-	-
PURPOSE	–	–	0.0027
inspect	0.434	0.235	0.0678
find leak	-0.799	0.360	0.0180
repair	0.195	0.235	0.4073
other	0	-	-
STOREFOAM	–	–	<.0001
wing	-1.669	0.344	<.0001
floor	-0.290	0.336	0.3898
enclosed	0	-	-
TEMPERATURE	–	–	0.0354
50 – 70 °F	0.885	0.339	0.0104
70 – 90 °F	0.168	0.184	0.3657
> 90 °F	0	-	-

Table 7. Final model for benzene in post-exposure breath.

Effect	Estimate	Std Err	p-Value
Intercept	2.224	0.266	<.0001
BZ_{air} ln(µg/m^3)	0.299	0.041	<.0001
BZ_{pre} ln(µg/m^3)	0.289	0.082	0.0006
SMOKED (yes)	0.385	0.155	0.0144
TRAVEL TIME (min)	-0.014	0.004	0.0009
RESPIRATOR	-0.605	0.204	0.0037
IRRITATION	0.324	0.142	0.0245
PEXERT	0.031	0.014	0.0322

Table 8. Final model for naphthalene in post-exposure breath.

Effect	Estimate	Std Err	p-Value
Intercept	0.155	0.414	0.7096
NAP_{air} ln(µg/m^3)	0.290	0.063	<.0001
IRRITATION	0.262	0.130	0.0466
PURGE	–	–	0.0020
blow	-0.133	0.289	0.6469
exhaust	0.567	0.344	0.1025
combo	0	-	-
HOWENTER	–	–	<.0001
did not	-0.385	0.223	0.0870
from top	-0.713	0.160	<.0001
from bottom	0	-	-
TEMPERATURE	–	–	0.0235
50 – 70 °F	-0.707	0.253	0.0063
70 – 90 °F	-0.164	0.134	0.2230
> 90 °F	0	-	-

FIGURES

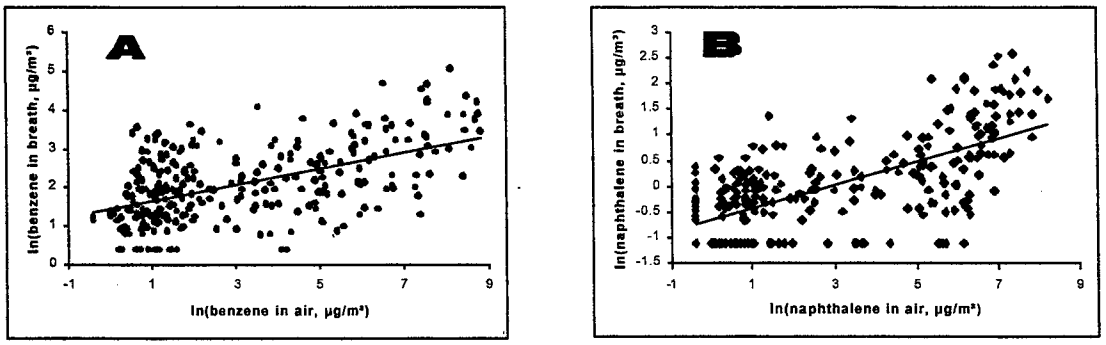

Figure 1. Relationship between benzene (A) and naphthalene (B) in air and in post-exposure breath for all subjects.

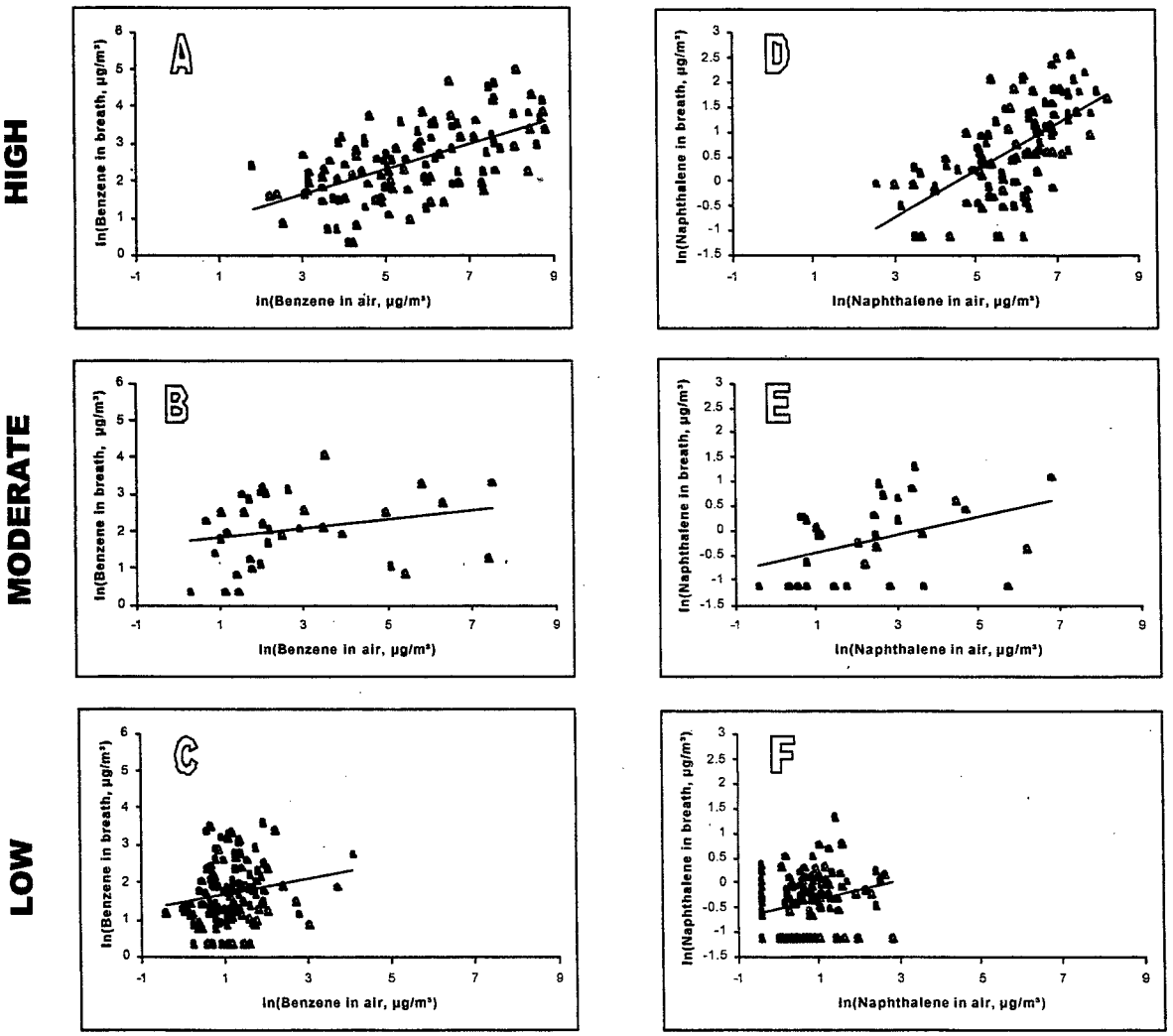

Figure 2. Relationship between benzene (2A-C) naphthalene (2D-F) in air and post-exposure breath by exposure category. Closed circles = smokers; open circles = nonsmokers.

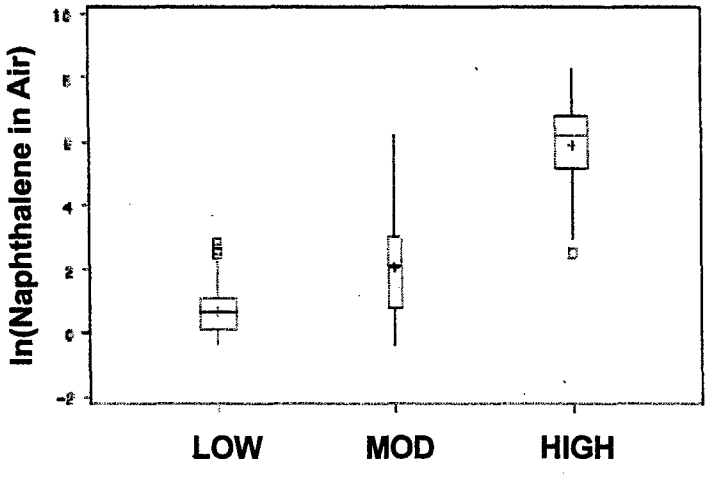

Figure 3. Comparison of distributions of (log-transformed) naphthalene in air by exposure category.

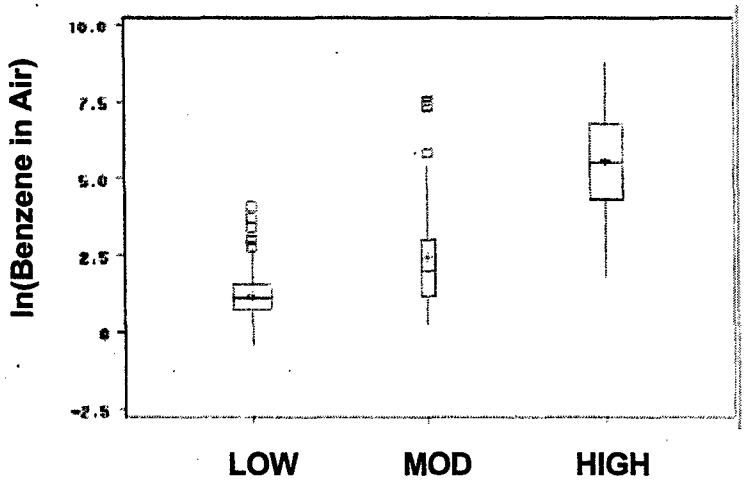

Figure 4. Comparison of distributions of (log-transformed) benzene in air by exposure category.

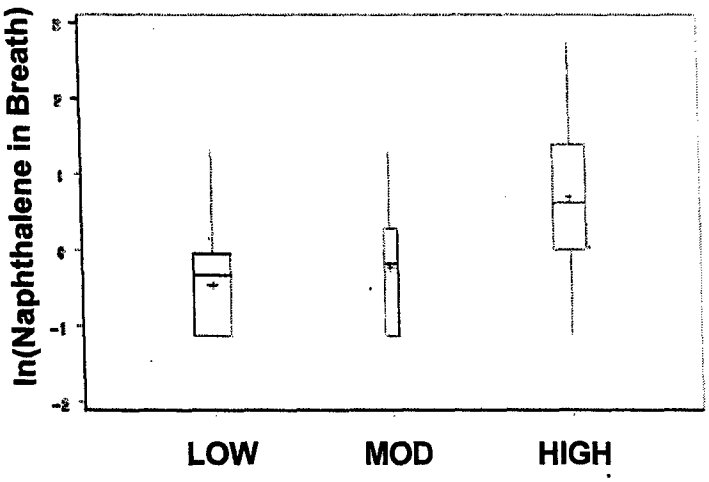

Figure 5. Comparison of distributions of (log-transformed) naphthalene in breath by exposure category.

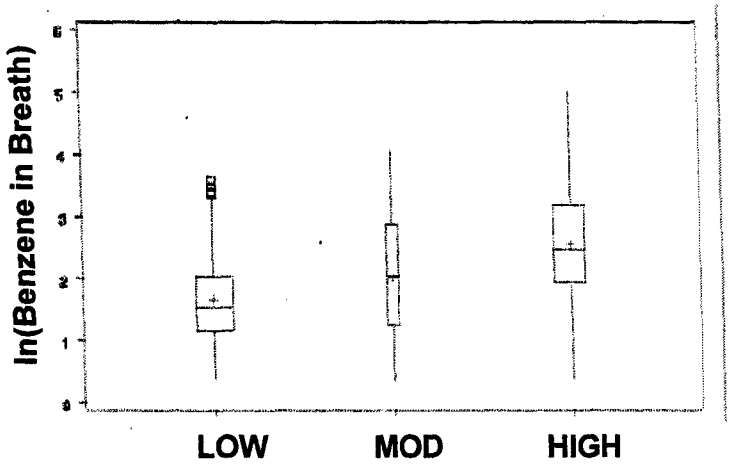

Figure 6. Comparison of distributions of (log-transformed) benzene in breath by exposure category.

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Non-invasive assessment of exposure to the jet fuel, JP8.

Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Exposure of Air Force personnel to the jet fuel, JP8 occurs in the occupational setting in the Department of Defense and civilian aviation. Research at Johns Hopkins University, supported by the Air Force Office of Scientific Research, is designed to evaluate whether Air Guard personnel experience adverse health effects as a result of chronic lifetime exposure to jet fuels. Air Guard personnel have an age distribution more typical of the general workforce and in our studies we plan to investigate the confounding variable, age. In order that these studies, which are in progress, can be related to the Air Force acute study directed by Lt. Col. Roger Gibson, JHU investigators attended three Air Force Bases. A common component in these studies is the estimation of the total body burden of jet fuel based upon the collection and analysis of exhaled breath. The JHU method for the collection and analysis of breath is different to the methods employed in the Gibson directed study. Therefore, one of the goals of this collaborative study was to compare and contrast these two protocols. Breath samples were collected from study subjects at Davis-Monthan, Langley, and Little Rock AFBs.

Methods:

Briefly, breath samples were collected from a relaxed breathing seated subject. A portion of the exhaled breath was collected for one minute at a constant sampling rate (80ml/min) onto a thermal desorption tube that contains proprietary adsorbent packing. The study subject was coached during breath collection with visual and audio aids. During this computer controlled breath collection, the tidal volume, the end-tidal concentration of carbon dioxide and the change in mouth pressure were monitored continuously. Additionally, the frequency of breathing and the steady-state concentration of carbon dioxide were monitored. The blood oxygen saturation and heart rate were also monitored for study subject during breath collection. Duplicate samples were collected. The thermal desorption tubes were transported to the laboratory and analyzed by two-stage thermal desorption fused silica gas chromatography with flame ionization detection. The details of this method of breath collection and analysis have been published in the peer-reviewed literature. The method is calibrated with known standards for some of the constituents found in JP8. Since the response factor for the flame ionization detector is dependent upon the number of carbon-hydrogen bonds in a molecule, it is possible to generate a complete JP8 profile. Analyses of individual compounds in JP8 and a profile for the complete JP8 were generated for each specimen collected. Additionally, attempts were made to estimate the route of exposure based upon the relative volatilities of the various constituents in JP8. At Davis-Monthan AFB, samples were collected pre- and post-exposure. At Langley and Little Rock AFB, samples were collected post-exposure.

Status:

All duplicate samples have been cataloged, analyzed, validated and entered into a spreadsheet. Final data in the form of g/m^3 and generation rates for each study subject

(pmol/kg.min) were submitted to the Air Force in January, 2001. The distribution of study subjects was 25 at Davis-Monthan AFB, 44 at Langley AFB, and 50 at Little Rock AFB. Of these, 71 subjects were nominally exposed and 48 were nominally unexposed.

Findings:

Nominally unexposed subjects at Davis-Monthan and Langley AFB had very similar concentration of JP8 profiles (mean 3.7 ± 1.8 mg/m^3) whereas nominally unexposed subjects at Little Rock AFB had concentration of JP8 profiles (mean 5.6 ±3.5 mg/m^3). The mean concentration of pre-exposed subjects at Davis-Monthan was 3.7 ± 1.2 mg/m^3. In all these subjects, the majority of the JP8 profile is derived from the least volatile components of JP8. The means for the nominally exposed subjects at Davis-Monthan AFB, Langley AFB, and Little Rock AFB were 40.1 ± 29.0 mg/m^3, 13.5 ± 18.6 mg/m^3 and 17.1 ± 13.7 mg/m^3 respectively. The ranges of levels of the concentration of JP8 varied significantly between nominally exposed subjects at each AFB. Moreover, the route of exposure appeared to be greater from inhalation at Davis-Monthan and Little Rock AFB as compared to Langley AFB.

Discussion/Conclusions:

The results from the analyses of exhaled breath collected during this study demonstrate conclusively that Air Force personnel are exposed to the jet fuel, JP8. Moreover, even the exhaled breath of nominally-unexposed subjects were found to contain quantifiable levels of JP8. Additionally, the exhaled breath of nominally exposed subjects who were performing similar duties had widely varying concentrations of JP8. These significant differences may be a reflection of the actual duties performed during the study period or the effective use of protection devices by some of the study subjects.

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Urinary Benzene, Naphthalene, 1- and 2-Hydroxynaphthalene as Biomarkers of Acute (Short-term) Exposure to JP8

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Introduction

This protocol is investigating the utility of urinary benzene, naphthalene, and hydroxynaphthalenes (HNs) as possible biomarkers of acute (short-term) exposure to JP8. Quantitative measurements of exposure to a chemical are critical for epidemiological studies that investigate the relationship between exposure and disease. However, inter-individual differences in the absorption, metabolism, and elimination of the chemical can be significant. Thus, measuring the internal dose can improve the accuracy and reduce misclassification in exposure variables. Of the hundreds of discrete chemicals in JP8, naphthalene and benzene are important aromatic constituents, which have been suggested as surrogate markers of JP8 exposure [2], [3], [4], [5].

Naphthalene and benzene have rapid elimination kinetics. Following inhalation, these compounds are absorbed into the blood. Some of the internal doses of these compounds are eliminated unchanged in breath and urine while the remainder is metabolized to products that are excreted in urine. Spot urine samples that are obtained at the end of the work-shift reflect exposure to benzene and naphthalene within the same day [1], [6]. Thus, urinary measurements will reflect only recent exposures. Despite this limitation, urinary biomarkers can provide better estimates of the individual internal dose compared to external exposure measurements, especially when dermal exposure occurs or where persons wear respiratory protection during the workday.

Methods

Urine samples (before and after the work period) were collected from Air Force personnel engaged in fuel cell operations and controls. Urinary naphthalene and benzene were measured based on the method of Waidyanatha S. et al. (2000). One-half ml of urine was transferred to a vial, containing 1 g of NaCl and internal standards. Benzene and naphthalene were adsorbed from the headspace by solid phase microextraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS) in the electron impact mode.

A new method was developed to measure urinary 1- and 2-HN (Serdar B. et al., in preparation). Two ml of urine were hydrolyzed enzymatically (using β-glucuronidase/sulfatase) along with a known amount of deuterated 1-HN. The analytes were extracted with ethyl acetate, and derivatized with Tri-Sil TBT (Pierce Chemical). The trimethylsilyl ethers were then analyzed by GC-MS in the electron impact mode.

Status

Urinary naphthalene and benzene have been measured thus far in pre- and post-exposure urine samples obtained from 63 subjects from Air Force personnel of Davis Monthan Air Force Base, Arizona (including 18 of the priority subjects). Urinary naphthalene and benzene will also be measured in the remaining priority samples. 1- and 2-HNs have been measured in pre- and post-exposure urine samples obtained from 99 priority subjects and will also be measured in the remaining priority samples.

Findings

Results of the analysis of urinary naphthalene and benzene

Urinary benzene and naphthalene were measured in pre- and post-exposure samples collected from 63 subjects at Davis Monthan Air Force Base. Based on the recent exposure reclassification, analyses were performed in samples from 29 high- and 19 low-exposed subjects after natural logarithmic transformation (using SAS system software, Cary, NC). Descriptive statistics are presented in Table 1. Results indicate that subjects in the high exposure group had significantly higher geometric mean urinary benzene and naphthalene ($p < 0.0001$ for both comparisons) compared to low exposed subjects following exposure. Geometric mean levels of benzene in pre-exposure urine samples were not significantly different between high and low exposed subjects ($p = 0.78$) while those for naphthalene were significantly different in these two groups ($p = 0.01$).

Urinary analytes in each exposure category were examined with respect to smoking status. Smokers had higher pre-exposure measurements of benzene in urine in both low and high exposure groups (p-values 0.02 and 0.01, respectively) when compared to nonsmokers within the same exposure group. However, post-exposure urinary benzene measurements were not significantly different between smokers and nonsmokers in either low or high exposure groups (p-values 0.42 and 0.40, respectively).

Pearson correlation coefficients were used to investigate the relationships between urinary analytes and exposure. Significant correlation was observed between the measurements of naphthalene in ambient air and post-exposure levels of naphthalene in urine among high exposed subjects ($r = 0.41$, $p = 0.03$). Similarly, benzene measurements in ambient air and post-exposure benzene in urine were significantly correlated among highly exposed subjects only ($r = 0.65$, $p = 0.0001$). Post-exposure urinary naphthalene and post-exposure urinary benzene levels were significantly correlated among highly exposed subjects ($r = 0.60$, $p = 0.0006$) but not among low-exposed subjects ($r = 0.091$, $p = 0.711$). Figure 1 shows the relationships between post-exposure urinary measurements and passive air measurements of benzene for the low- and high-exposure groups. Figure 2 shows the same relationships for naphthalene.

Results of the analysis of urinary 1- and 2-hydroxynaphthalene

Urinary 1-, and 2-HN were measured in samples from 99 subjects. Using the recent exposure reclassification, measurements of 75 subjects (43 low- and 32 high-exposed) were used for statistical analyses after natural logarithmic transformation. Descriptive statistics are presented in Table 2. Results indicate that post-exposure geometric mean urinary 1- and 2-HN measurements were significantly higher ($p < 0.0001$ for both comparisons) in the high-exposure group compared to the low-exposure group. Mean levels of 1- and 2-HN in pre-exposure urine samples were not significantly different between exposure groups (p-values 0.06 and 0.74, respectively). Urinary analytes in each exposure category were examined with respect to smoking status. In the low-exposure group, measurements of 1-HN ($p = 0.003$) and 2-HN ($p = 0.005$) in pre-exposure urine samples were significantly higher in smokers when compared to nonsmokers. Similarly, measurements of 1-HN ($p < 0.0001$) and 2-HN ($p = 0.004$) in post-exposure urine samples were significantly higher in smokers when compared to nonsmokers in the low exposure group. In the high-exposure group, geometric mean levels of urinary 1-, and 2-HN in pre-exposure urine samples were significantly higher in smokers when compared to nonsmokers ($p = 0.002$, and $p < 0.0001$, respectively), but this difference was not observed in post-exposure urine samples (p-values 0.29 and 0.24, respectively). When log-transformed values are plotted (Figures 3 and 4) marked effect of exposure and lesser effects of smoking were observed.

The relationships between HNs in urine and naphthalene in air were investigated using Pearson correlation coefficients. The correlations between 1- and 2-HN in pre-exposure urine samples were significant in all exposure groups ($p < 0.0001$ for all comparisons). The correlations between pre-and post-exposure measurements of each HN were significant only in the low-exposure group ($p < 0.0001$). In the high-exposure group, naphthalene measurements in air were significantly correlated with both 1-HN ($r = 0.72$, $p < 0.0001$) and 2-HN ($r = 0.43$, $p = 0.02$) measurements in post-exposure urine samples. Significant correlation was not observed in samples of the low-exposure group. Figures 5 and 6 show the relationships between post-exposure urinary HN and naphthalene in air for the low- and high-exposure groups.

Discussion/Conclusions:

The relationships between levels of airborne and urinary benzene and naphthalene (Figure 1 and 2) confirm a monotonic trend among exposed subjects but not among controls (low-exposure group). Similarly, the relationship between levels of airborne naphthalene and urinary HNs (Figures 5 and 6) confirm a monotonic trend among high exposed subjects but not among controls (low-exposure group). Our results also indicate that the levels of urinary benzene and HNs are higher among control subjects who are smokers. However, the effect of smoking becomes less significant as exposure increases.

Measurements of urinary analytes in samples obtained at the end of the work-shift were significantly correlated among high-exposed subjects but not among low-exposed subjects. These results indicate that there is a common source of exposure to benzene and naphthalene during the work-shift among exposed subjects. It is reasonable to conclude that the source of exposure was JP8. Air and pre-shift urine samples were not significantly correlated, indicating that the exposures to benzene and naphthalene before the work-shift did not have a common source.

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Table 1. Geometric means (in μg/l) of urinary naphthalene and benzene, categorized by exposure group. (GSDs shown in parentheses).

Urinary analyte	Low exposure (n = 19)	High exposure (n = 29)
	GM (GSD)	GM (GSD)
Pre-exposure benzene	0.664 (2.09)	0.716 (2.63)
Pre-exposure naphthalene	0.005 (2.94)	0.013 (3.16)*
Post-exposure benzene	0.669 (1.57)	1.812 (2.68)**
Post-exposure naphthalene	0.005 (4.07)	0.212 (6.39)**

* $p<0.05$ for test of equal geometric means against the low exposed group

** $p<0.0001$ for test of equal geometric means against the low exposed group

Table 2. Geometric means (in μg/l) of urinary 1- and 2-HN, categorized by exposure group. (GSDs shown in parentheses).

Urinary analyte	Low exposure (n = 43)	High exposure (n = 32)
	GM (GSD)	GM (GSD)
Pre-exposure 1-HN	1.851 (3.16)	3.209 (3.81)
Pre-exposure 2-HN	3.152 (2.84)	3.445 (3.41)
Post-exposure 1-HN	1.365 (3.97)	9.944 (2.38)**
Post-exposure 2-HN	2.508 (4.30)	17.975 (2.91)**

*$p<0.05$ for test of equal geometric means against the low exposed group

**$p<0.0001$ for test of equal geometric means against the low exposed group

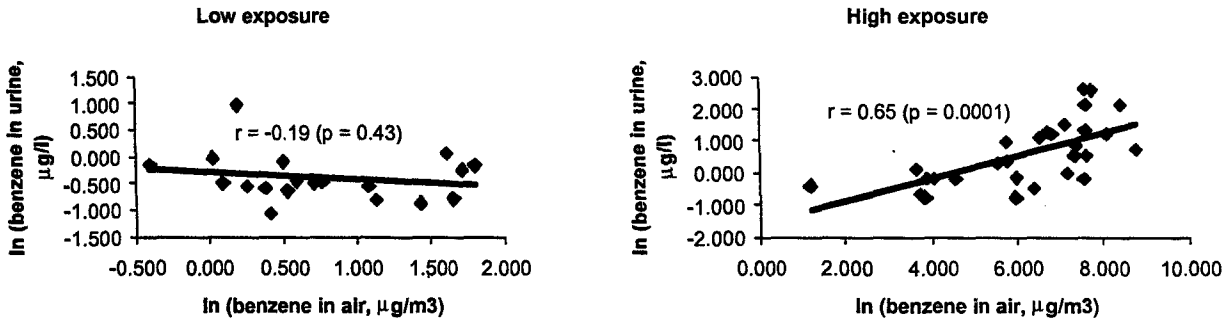

Figure 1. The relationships between the logged values of benzene in urine (after work) and benzene in air ($\mu g/m^3$). Pearson correlation coefficients and p-values shown.

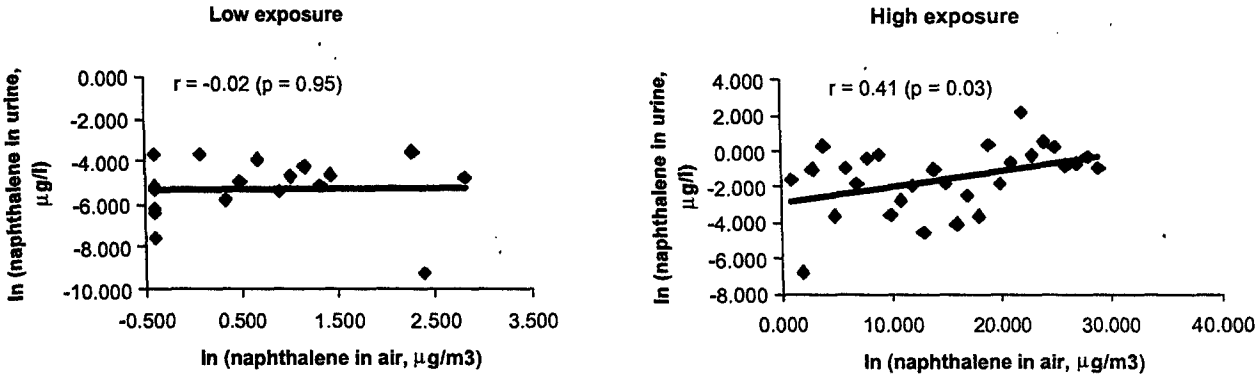

Figure 2. The relationships between the logged values of naphthalene in urine (after work) and naphthalene in air ($\mu g/m^3$). Pearson correlation coefficients and p-values shown.

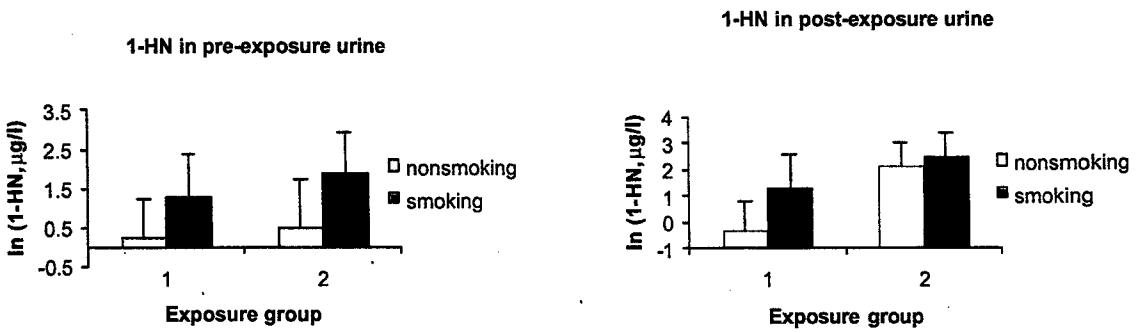

Figure 3. Mean levels of urinary 1-HN in pre-and post-exposure urine samples aggregated by exposure category (low = 1 and high = 2) and smoking status. Means and SEs of log-transformed data shown

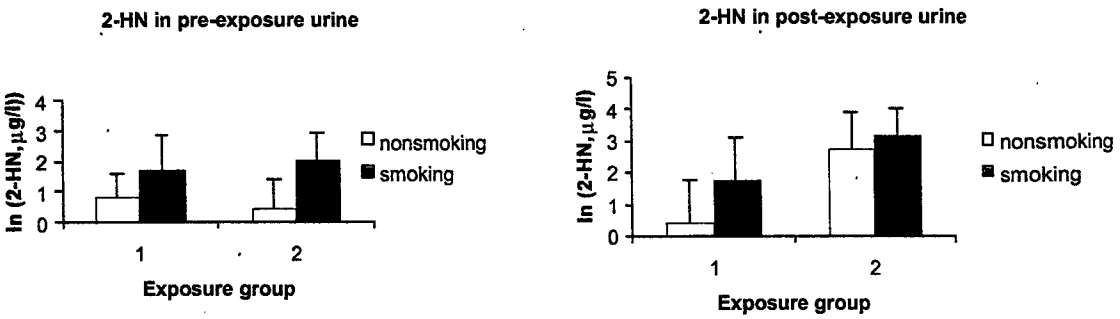

Figure 4. Mean levels of urinary 2-HN in pre-and post-exposure urine samples aggregated by exposure category (low = 1 and high = 2) and smoking status. Means and SEs of log-transformed data shown

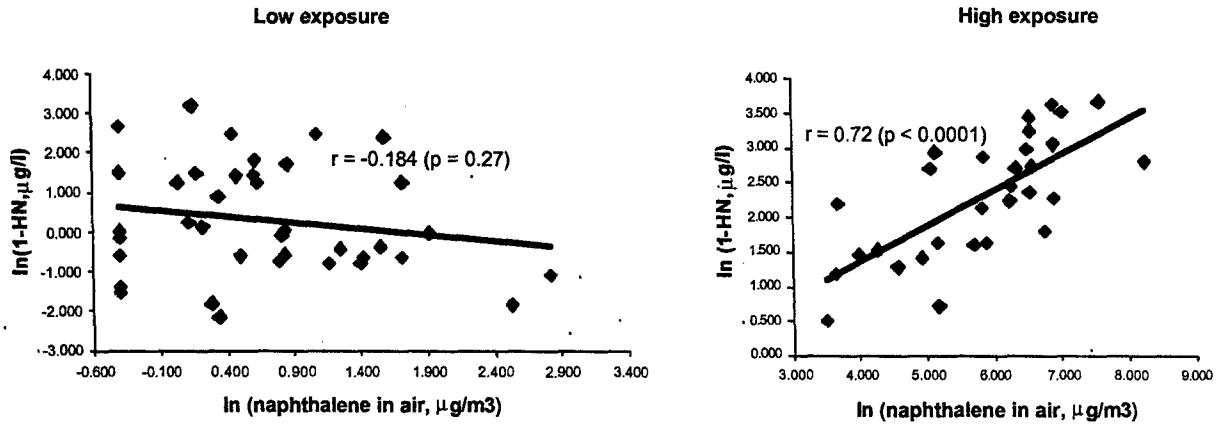

Figure 5. The relationship between the logged values of post-exposure urinary 1-HN (µg/l) and passive measurements of naphthalene in air (µg/m^3). Pearson correlation coefficients, and p-values shown.

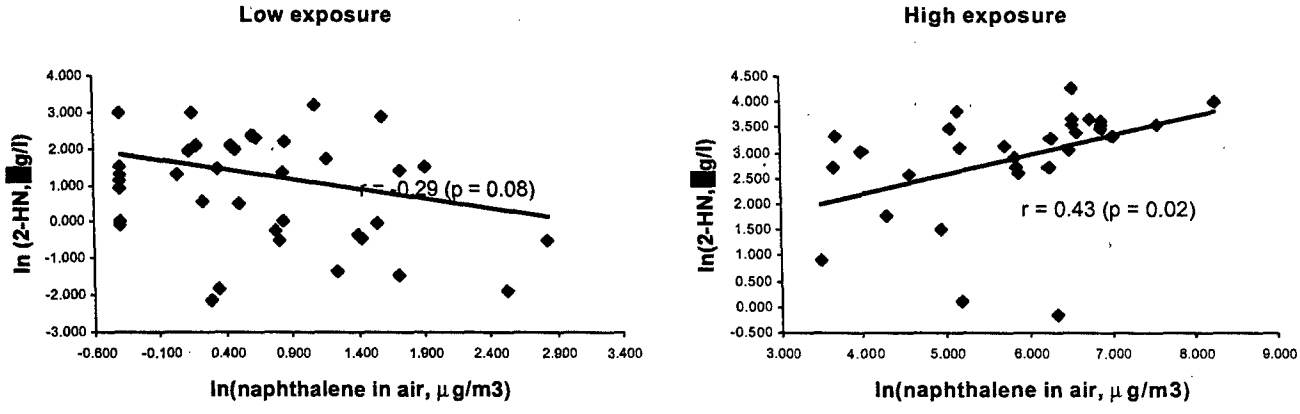

Figure 6. The relationship between the logged values of post-exposure urinary 2-HN (µg/l) and passive measurements of naphthalene in air (µg/m^3). Pearson correlation coefficients, and p-values shown.

RESULTS and DISCUSSION (Neurobehavioral)–Interim Report (August 16, 2001)
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SF-36 Standardized Assessment of Physical and Mental Health Symptoms

The SF-36 was administered to identify self-reported symptoms of physical and mental health (Ware, et al., 1988; Ware and Sherbourne, 1992). The mean number of symptoms of the HIGH exposed group did not differ significantly from the LOW (controls) on the physical functioning scale, but the HIGH group did have significantly more symptoms on the remaining 8 scales. The Bodily Pain and General Health scale differences remained significant after a highly conservative Bonferroni correction for multiple comparisons (9 measures).

While an SF-36 result is not sufficient for a clinical diagnosis of emotional or psychological distress, it is one measure used in the development of such diagnoses and would be expected to identify severe mental or emotional problems in most cases. A review of the SF-36 results did not reveal any individual scores indicative of emotional stress so severe as to suggest that any Participant's neurobehavioral data should be excluded from the analysis for reasons of potential bias.

BARS Neurobehavioral Assessment

Seven standard neurobehavioral tests presented in the Behavioral Assessment and Research System (BARS) (Anger et al., 1996) provided 12 primary measures of motivation, learning, memory, attention, response speed and coordination before (PRE-shift) and after (POST) the 4- to 6-hour work period. Results on 1 or more tests were eliminated for 38 Participants because of notes from the Examiners about Participants not following instructions or because the scores were so extreme that they would have added a large amount of variability to the statistical analyses. For most analyses of the neurobehavioral test results, there were 117 Participants in the HIGH group and 165 in the LOW group. The MOD group was not included in the analyses because their number was small and because they overlapped the exposure distributions of the other groups, which could have blurred distinctions between exposure groups rather than sharpen them. The HIGH and LOW groups differed significantly in age, gender, and scores on the Armed Forces Qualifying Test (AFQT) obtained at entry into military service (i.e., before fuels exposure began) (Table1). Age, gender and AFQTgen scores are known to affect performance on neurobehavioral tests, and multiple correlational analyses demonstrated significant correlations of these measures with the neurobehavioral measures in the study Participants. Therefore, age, gender and AFQT scores were entered into an Analysis of Covariance (ANCOVA) to eliminate the variance associated with these measures.

Table 1. Age, Gender (%male) and Armed Forces Qualifying Test (AFQT) scores in HIGH (Fuel-Exposed) and LOW (controls).

	Age	Gender	AFQTgen
HIGH (fuel-exposed)	24.4	94% males	56
LOW (controls)	26.3	73% males	65

HIGH vs. LOW comparisons at PRE

On the morning of the test day prior to that day's exposures (PRE), HIGH group performance was inferior to LOW group performance on 9 of the 12 primary neurobehavioral measures. ANCOVAs revealed that the HIGH group had significantly lower performance on the Digit Span forward (p=0.03) and backward (0.006), Symbol Digit latency (p=0.003), and Tapping/preferred hand trials 1 and 2 (both p=0.04), as compared to the LOW group (Table 2). The difference on the Symbol Digit, arguably the most sensitive test for non-resolving neurotoxic effects, remained significant after a highly conservative Bonferroni correction for 12 comparisons (the 12 primary neurobehavioral measures). This consistent trend of lower performance by the HIGH group following correction for age, gender and AFQT scores, is strongly indicative of a carry-over or non-resolving effect of working in fueling jobs. In initial analyses, there was no evidence of correlation of these deficits with breath or passive naphthalene or benzene exposure measures at PRE (morning), although there was evidence of an association between PRE naphthalene exposure and Match to Sample performance (accounting for 1 % of the variance in multiple regression analyses). (Additional exposure measures that were collected but have not been available for these analyses could affect this result.) Such correlations would tend to support carry-over of exposures from the days before. In the present absence of such correlations, the results support but cannot fully demonstrate the existence of a non-resolving effect that would continue after fuels exposure stopped. Neurobehavioral performance differences between PRE (morning) and POST (afternoon) were also seen, but ones that are different from those found at PRE (Table 2 and below). This suggests a different effect than seen at PRE which would support a hypothesis of non-resolving differences, an issue that merits further exploration.

Table 2. Probabilities on Neurobehavioral Measures that Differed Significantly between HIGH (Fuel-Exposed) and LOW (Control) Groups, Significant Regression Analyses for Naphthalene, and Percent Variance Accounted for by the Naphthalene Exposures.

	Digit Span Forw/Back	Symbol Digit	Tap pref 1	Tap pref 2	Tap alt	ODTP correct	Match to Sample
PRE	0.03/0.006	0.003	0.04				
PRE-POST				0.01	0.004		<0.0005
Naphthalene regression				0.02		0.007 passive	<0.0005 passive
% variance				1%		2%	5%

PRE vs. POST Comparisons

Comparisons between the HIGH and LOW groups at POST exposure employed the same covariates of age, gender and AFQTgen. To account for performance differences and the effort expended during the work day, PRE performance on the same neurobehavioral test and PEXERT, a measure of self-reported physical and mental work and the stress from that work, were included in the POST analyses. This revealed significant comparisons on three measures: Match to Sample score (p<0.0005) and Tapping for preferred hand trial 2 (p=0.01) and alternating hands tapping (0.004). The Match to Sample and alternating hand Tapping differences remained significant after a conservative Bonferroni correction for multiple comparisons.

Multiple regression was used to determine what proportion of neurobehavioral test performance variance could be explained by the exposure variables after age, gender, AFQTgen, PEXERT and PRE neurobehavioral test performance were entered into the analyses. Using only the exposure measures available on all (including MOD) Participants for these analyses (viz., naphthalene and benzene passive and breath), regression analyses for three neurobehavioral measures were significant. Passive naphthalene exposure (from day-long samples) was associated with inferior performance on Tapping/preferred hand trial 2 ($p=0.02$), Match to Sample ($p<0.0005$), and ODTP correct ($p=0.007$). The variance accounted for by the naphthalene exposures was 1% for Tapping trial 2, 2% for ODTP correct, and 5% for Match to Sample (Table 2). By comparison, age in this comparatively young group accounted for approximately 2% of the variance. This result is indicative of an acute exposure effect on memory for numbers and patterns following a delay, and response speed and coordination.

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Summary for Website – Neurobehavioral Tests

W. Kent Anger, PhD and Dan Storzbach, PhD (Oregon Health Sciences University)

A series of 7 neurobehavioral tests in the Behavioral Assessment and Research System (BARS) were administered to all study participants at the beginning (PRE) and end (POST) of a 4- to 6-hour work period. To determine Participants' recent history of physical and mental health symptoms, the SF-36 standardized questionnaire assessment was administered. At PRE, the HIGH exposed group reported significantly more symptoms on 8 out of 9 scales than did the LOW (control) group. The increased symptom reports on the Bodily Pain and General Health scales remained significant after a conservative Bonferroni statistical correction for multiple comparisons.

An objective assessment of memory, attention, learning, motivation, response speed and coordination was provided by 12 primary measures extracted from the 7 standard neurobehavioral tests. The HIGH and LOW groups differed on factors that affect neurobehavioral tests, including age, gender, and scores on the Armed Forces Qualifying Test (AFQT) and "PEXERT" which reflected self-reports of work effort and stress at the end of the test day. Analysis of covariance (ANCOVA) which corrected for age, gender and AFQT scores revealed that the HIGH group had significantly lower performance on the Digit Span forward and backward, Symbol Digit latency, and Tapping tests, as compared to the LOW group at PRE, in the morning before exposures began. These are differences in recall of short number sequences, a coding task benefiting from good recall and rapid responding, and response speed and coordination. Regression analyses revealed a lack of correlation of these deficits with breath or passive naphthalene or benzene exposure measures at PRE (morning), suggesting that these differences resulted from carry-over of exposures from prior days. Whether the lower scores are non-resolving and might continue after fuels exposure stops, merits further exploration.

Neurobehavioral performance differences between PRE (morning) and POST (afternoon) were also seen, but the affected measures are different from those found at PRE, suggesting a different effect than seen at PRE. PRE-POST differences in Match to Sample (requiring memory of a pattern of black and white squares) and alternating hands Tapping were statistically significant, with HIGH declining more than LOW. Multiple regression was used to determine what proportion of neurobehavioral test performance variance could be explained by the exposure measures after correcting for age, gender, AFQT, PEXERT and PRE neurobehavioral test performance in the statistical analysis. Using only the exposure measures available on all participants for these analyses (viz., naphthalene and benzene passive and breath), regression analyses for three neurobehavioral measures were statistically significant. Passive naphthalene exposure was significantly associated with performance on Oregon Dual Task Procedure (ODTP) correct, Match to Sample, and Tapping trial 2. Of these, the variance accounted for by the naphthalene exposures was 1% for Tapping, 2% for ODTP, and 5% for Match to Sample. By comparison, age in this comparatively young group accounted for approximately 2% of the variance. This result is indicative of an acute exposure effect on memory for numbers and patterns following a delay, and response speed and coordination.

Eyeblink Conditioning Response Test used to Assess Performance in JP8 Exposed Air Force Personnel
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Extensive research during the past 10-15 years has demonstrated that eyeblink classical conditioning (EBCC), easily learned by virtually all human population groups (including infants), can be significantly modulated by aging, acute stress, pharmacological challenges, or the onset of certain neurological disorders such as Alzheimer's or Parkinson's disease. This sensitivity suggests the EBCC could also be used to assess neurobehavioral deficits resulting from neurotoxic exposures. In addition, the neural circuitry subserving sensory input, motor output, and associative learning aspects of EBCC is well known. Different testing paradigms can be used to test individual neural pathways and more clearly localize the areas affected by putative neurotoxic agents. For these reasons, EBCC is an appropriate tool for neurobehavioral risk assessment, and it was used in the evaluation of neurobehavioral integrity in Air Force personnel with occupational exposure to JP8.

The human EBCC paradigm used in the present study is particularly sensitive to deficits in cerebellar functions. This delay paradigm involves the presentation of a salient (tone) conditioned stimulus (CS) preceding and concurrent with the delivery of a corneal surface airpuff unconditioned stimulus (US), inducing a reflexive eyeblink response (unconditioned response or UR). It has been consistently demonstrated that humans, non-human primates, and many laboratory small animal species rapidly acquire an association between the CS and the elicitation of a UR, such that mere presentation of the tone reliably results in the eyeblink response (conditioned response or CR). The CR is defined by its occurrence within a temporal window that excludes incidental/anticipatory eyeblinks (classified as alpha responses) or those eyeblinks elicited directly by the US and are thus classified as URs. Previous reports suggest that this delay conditioning EBCC paradigm minimally requires integrity of several brainstem auditory and motor relay centers, specific nuclei in the cerebellum, and associated neural pathways. Research indicates no higher brain processes are required for the acquisition or retention of this particular learning task. The delay paradigm of EBCC was employed to assess changes in basic, or reflexive, neurobehavioral response patterns as a result of JP8 exposure.

Methods:

A total of 126 male (80%) and female (20%) subjects (fuel-exposed and non fuel-exposed controls) were tested for EBCC. For the purpose of the current analyses, subjects were categorized into low (control) and high exposure groups, based on self reports of occupational responsibilities and post-exposure measures of naphthalene. The high exposure group was primarily comprised of personnel who routinely participated in aircraft fuel tank cleaning and repair, and other avionics maintenance tasks requiring significant daily exposure to JP8. Those in the low exposure group (matched for multiple factors) were employed for similar time periods on the same USAF bases, but reported little to no known exposure to JP8.

EBCC Paradigm: EBCC equipment was procured from San Diego Instruments, Inc. (San Diego, CA). Following a brief orientation, subjects sat comfortably in a chair viewing a silent

movie on a TV/VCR in order to minimize head and eye movement. Subjects were fitted with stereo earphones that presented a 1000 Hz, 80 dB tonal CS, against a background 70 dB white noise. They also wore an adjustable "sunglasses-like" headpiece fixed with an airpuff delivery nozzle and an infrared photocell transducer to measure occurrence and amplitude of the eyeblink response. Voltage changes in the transducer were input to a microprocessor. The airpuff nozzle was positioned 1-2 cm from the left cornea, and delivered a 100-ms, 3-5 psi airpuff of room air (US) from a small air generator unit attached by flexible tubing to the headpiece. A 30-sec subject acclimation period preceded baseline trials. Baseline trials consisted of 13 US-only trials [5-10 sec variable intertrial interval (ITI)], and provided an average, individual maximum eyeblink amplitude for each subject. EBCC consisted of 63 trials with a variable ITI of 10-15 sec; 56 trials presented CS-US pairings while the remaining 7 trials presented CS alone. Total testing time was 18-19 min. Ten percent of subjects were administered a brief hearing test to ensure that occupational exposures had not increased auditory threshold for a 1000 Hz tonal CS. No subjects had to be eliminated due to hearing deficits.

Data Reduction: Only eyeblinks with an amplitude of at least 20% of an individual subject's maximal eyeblink were counted as either URs or CRs. Thirty-two subjects were eliminated because they did not meet a criterion of at least 30% URs. Since the UR is considered a reflexive response, a score of less than 30% suggests a problem with either the equipment or the subject. The most common scenarios were drowsy subjects and an improper fitting of the eyeglasses such that the airpuff was not delivered to the correct area of the eye. In some cases, the eyeglass positioning was altered by the subject moving or changing positions. Twelve subjects were eliminated because they did not meet the criterion for either the low or high exposure groups. They were part of a heterogeneous moderate exposure group that was not included in these analyses. Five participants were not included in the final data analyses because it is suspected the they received unintentional, albeit minimal, exposure to JP8 vapors due to off-gassing from other exposed participants. A few additional subjects were excluded because they had too many bad trials where a bad trial is defined as one where the eyeblink was anticipatory or incidental. Final analyses were conducted on a total of 74 subjects, 59 male and 15 female. All but 1 female was in the low exposure group. No gender differences were found in the low exposure group, so gender was not considered a variable in further analyses. The number of subjects per Air Force base was: Pope = 16, Seymour Johnson = 9, Langley = 18, Little Rock = 14, Davis Monthan = 9, and Hurlbert = 8.

Statistical Analyses: A two-way repeated measures ANOVAs (exposure group [2] x test block [7]) was conducted separately for the morning session and the afternoon sessions for the dependent variable of percent of CRs. Averages for the morning and afternoon sessions were calculated for each of the remaining dependent variables: latency to onset of CR, latency to peak of CR, and latency to onset of UR. Single factor ANOVAs were completed to determine the effects of low versus high exposures for these three variables.

Status:

The data for individual subjects has been processed and is ready to be added to the database. Our data is formatted for joint analyses, and subsets of the final scores have already been shared with co-investigators.

Findings:

For the morning session, a statistically significant main effect ($p < 0.05$) was found for exposure group for measures of: percent CRs, CR peak latency, and CR onset latency, but not for UR onset latency. Personnel in the high exposure group showed fewer CRs than those in the low exposure group. Shorter latencies for CR peak and CR onset were also observed in the high versus low exposure groups. No statistically significant exposure-based differences were found for the afternoon session.

Discussion/Conclusions:

Overall, the most apparent group differences occur in the morning session, suggesting a subchronic/chronic effect. There does not appear to be an obvious acute effect as there are no group differences in any of the measures for the afternoon session. While the observed differences do not provide enough information to conclude that there would be obvious group differences in overall capabilities or job performance, they do provide an overall indicator of subtle neurological deficits. Based on the neuroanatomical requirements of the eyeblink response, the deficits are most likely due to effects in the cerebellum. This is a brain structure that is known for its contributions to overall fine motor coordination, equilibrium and balance, and associative learning as occurs in the eyeblink paradigm. Thus, one would expect to see mild to moderate deficits in these areas with changes at the cerebellar level such as those evidenced in the present research. The specific findings of this study suggest a notable deficit in basic associative learning, but not in the recall of the task as indicated by no group differences in the afternoon session. The finding of fewer CRs indicates more trials were required to learn the association in the high versus low exposure groups. Second, the shorter latencies for CR onset and peak may suggest a deficit in the timing mechanism that controls this reflexive response. For instance, the CR can occur anywhere between 100-400 msec, however, the closer the response is to 400, the better the timing mechanism, or the better the learning. Indeed, it has been demonstrated that well-trained subjects will have blink latencies that are closer to the onset of the US (airpuff to the cornea) than to the CS(tone). This is likely a result of the advantage timing accuracy affords as a protective mechanism against the US. In the present case, the exposed subjects are responding earlier in this temporal window than the controls, thus indicating poorer performance. While the latency to UR onset is not a direct measure of learning, the group differences may be related to an overall disinhibition (or lack of inhibition) within the response system. This hypothesis could be directly addressed by testing extinction and blocking response patterns using EBCC. Also, since the results clearly suggest deficits in cerebellar circuits related to the eyeblink response and classical conditioning, future investigations should include evaluation of functional neuroanatomical analyses as can be accomplished with functional magnetic resonance imaging (fMRI).

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Postural Balance Measurements
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Very little research has been published on the quantitative measurement of neurological effects of exposure to JP8 jet fuel. JP8 jet fuel's acute toxicological effects are expected to resemble those of kerosene, a CNS depressant. A 1996 report from the U.S. National Research Council's (NRC) Committee on Toxicology noted the lack of quantitative research on CNS and associated performance measurements of workers with exposure to jet fuel vapors. The NRC report recommended more research using quantitative measurements on the effects of jet fuel vapors to the nervous system.

Postural balance measurement provides a unique "biological marker" of environmental chemical-associated changes in functional aspects of the nervous system. The technique has been tested, validated and found to be sensitive enough to detect significant changes in body balance with a reference solvent (ethanol) level as low as 0.02% blood alcohol level. In a previous study from our laboratory with children with chronic exposure to environmental lead, we found an increase in postural sway of 162 children to be significantly correlated with their blood-lead level. In a study of 37 pesticide applicators, a significant increase in postural sway implicating proprioceptive impairment was noted. In another study with 30 Air Force maintenance personnel, we reported significant association between subjects' postural sway and chronic exposure to jet fuel. This method is a relatively simple, sensitive, non-invasive and reproducible technique to evaluate subtle neurological dysfunction.

This investigation will address acute JP8 jet fuel exposure in a healthy workforce to determine if an effect is evident in their postural balance measurements. The specific aims of the study are: (a) to compare postural balance of air force mechanics before and after acute exposure to JP8, (b) to determine if there is a relationship between chronic exposure to jet fuel constituents and changes in postural sway, and (c) to measure performance-based postural balance outcomes in the exposed population.

Methods:

Postural sway measurements are conducted with an Advanced Mechanical Technology, Inc (AMTI) "AccuSway Plus" portable measurement platform and a laptop computer. The platform provides direct outputs for forces in the vertical direction (Fz), horizontal directions (Fx and Fy), and moment around the x-axis (lateral), moment around the y-axis (anterior-posterior) and moment around the vertical z-axis. The data is analyzed with the KineLysis Software developed by the University of Cincinnati (All rights reserved 2000). This software calculates the x-y coordinates of body's center of pressure for each test. Sway Area and length are used to characterize the sway patterns obtained. Total area (cm^2) of sway (SA) is the area enclosed within the envelope of the outer perimeter of the x-y plot of the center of pressure. Total length (cm) of sway (SL) is determined by the distance, traversed by the center of pressure during the test period. The technique used to measure postural sway quantifies movement patterns of the body's center of pressure associated with body sway as an indirect assessment of the central nervous system effect. The test indirectly measures the effect of proprioceptive, visual, and vestibular systems on the maintenance

of postural balance. As postural control systems are compromised, changes in the sway pattern can be quantified through mapping of increased postural sway.

To measure postural sway, all subjects (89 males and 13 females) performed a series of four separate 30-second tests in two separate trials (Trial 1 and Trial 2). For Trial 2, the tests were conducted in the reverse order. Each test was developed to test separate portions or combinations of the subject's postural control systems. Exact foot placement was maintained between tests by drawing an outline of the subject's feet on a paper cover taped onto the force platform. The subject's foot angle was maintained at 30 degrees. This angle was determined by use of a wedge during alignment, prior to testing. The test included the following conditions:

(EO): eyes open, standing on bare platform -- This tested the collective effect of the visual, proprioceptive, and vestibular systems controlling postural sway.

(EC): eyes closed, standing on bare platform -- This removed the visual system and therefore tested the proprioceptive and vestibular systems.

(FO): eyes open, standing on 4-inch foam placed over the platform -- This test destabilized the proprioceptive system and therefore tested the visual and vestibular systems.

(FC): eyes closed, standing on 4-inch foam placed over the platform -- This test removed the visual system and destabilized the proprioceptive system that allowed the vestibular system to act as the primary control of postural sway.

Two bending tests were also performed, eyes open (BO) and closed (BC) , which were performed to determine ability and rate of recovery from the bending of torso. These tests were the same as above except the subject bent over, torso at 90 degrees, held the position for five seconds then returned to the erect position. This made a total of ten tests. These tests were carried out once in the morning before (Pre-test) the subject started his/her work and again after about 4 hours of work (Post-test). An increase in the sway variables implies poorer postural balance.

Data Analysis:

The data from EO, EC, FO, and FC, conditions were used for statistical analysis after averaging the two trials. BO and BC had only one trial. The dependent variables for the regression model were the natural log of post sway area (SA) and natural log of post sway length (SL). The independent exposure variable used was the natural log of the passive naphthalene. The subjects were placed in HIGH, (n = 44) MODERATE (n = 11) and LOW (n = 47) exposure groups according to their passive naphthalene exposure levels. Since there were only 11 subjects in the MODERATE group we merged this group into the HIGH group. The mean (SD) of age for the HIGH+MODERATE and LOW groups was 25.05 (5.33) yrs. and 27.8 (5.8) yrs. respectively. The ranges of age for these two groups were 18.7 to 40.9 yrs. for the HIGH+MODERATE (HIM) group and 18.8 to 43.3 yrs. for the LOW group. The postural balance responses of these two groups were compared with an unexposed (OLDER UNEXP) group (n = 26) from another study from our laboratory. The mean (SD) age of subjects from the OLDER UNEXP group was 34.4 (7.97) and the range was 21 to 57.1 years. Since with age, postural balance deteriorates a comparison with older group will give us a more conservative result. The JP8 group's postural sway (log sway area) response as a whole (HI+MOD+LOW) was also compared with another existing database of unexposed subjects (YOUNGER UNEXPOSED) from our laboratory (10 males + 10 females). The mean (SD) age of male and female YOUNGER UNEXPOSED subjects were 25.8 (2.8) yrs. and 25.7 (3.56) yrs., respectively, which were much closer to the mean age of the JP8 subjects.

An analysis of covariance (ANCOVA) using backward elimination of insignificant covariates was used to determine the effect of JP8 exposure to postural sway measurements. Two statistical models were tested with dependent variables as Pre and Post sway variables. The Post sway variables were tested with and without Pre sway variables in the model. All the models were tested separately for EO, EC, FO, FC, BO and BC conditions. The SAS procedure PROC GLM was used for the analysis of the covariance. The independent variables that remained after this process were identified as cofactors in the regression model. Other independent variables included the pre SA, pre SL and the day of week tested. Covariates included body mass index (kg/cm), age (years), months on the job, gender (M or F), race (White, Black, Native American, Asian/Pacific Islander, Hispanic, other), alcohol use (alcohol use x average days per week x average drinks per day = 0-50), current smoker (Y or N), if the subject smoked on day of testing (Y or N), mental exertion (1-9), physical exertion (0-24), and time the subject left their job until the postural sway was tested (minutes). For all variables, a one-tailed alpha = 0.05 was used to test the hypotheses of no effect due to exposure since the postural balance outcome measures were hypothesized to be increased with increased exposure.

Status:

The postural balance response results provided are based on exposure parameter of passive naphthalene. We will be interested in redoing our analysis with other exposure variables such as breath levels of solvents and other constituents of the jet fuel and also other biomarkers. In addition, it will be worthwhile to conduct further analysis dealing with postural balance and other performance variables such as GASH (Dr. Anger), Eye Blink (US. Navy)., Gene (Dr. Butler) etc.

Findings:

Based on ANCOVA analysis after controlling for cofactors, the post-log sway length was found to be significantly associated with the variable of acute exposure to natural log of passive naphthalene for the Eyes closed no foam (FC) and the Eyes closed bending (BC) test conditions only. Cofactors which were found to be significant were age and gender.

Table 1. Post Sway response and its association with Natural log Passive Napthalene (ln N) without Pre sway in the model

Test	Dependent Variable	Independent Variables	Parameter Estimate	Standard Error	P values (or tailed)	Model R^2
EC	Ln SL	Intercept	3.55	0.14		0.18
		Ln N	0.015	0.0094	0.05	
		Age	0.015	0.0048	0.0017	
BC	Ln SL	Intercept	4.42	0.14		0.24
		Ln N	0.012	0.007	0.047	
		Age	0.0064	0.0034	0.03	
		Gender (F)*	-0.14	0.06	0.009	

* Sway response was higher in males than in females

It was noted that the significant relationship for post log sway length with exposure variable disappeared once the pre-sway variable was put in the model. The post sway data from LOW and HI+MOD groups were compared with an OLDER, UNEXPOSED group while controlling for cofactors. This analysis showed that least squares means of post sway area responses of both JP8 groups for the Eyes closed on foam (FC) test were significantly higher than that for the OLDER UNEXPOSED group (p value from Dunnett-Hsu test: 0.0011). The pre log sway area and pre log sway length responses of HI+MOD JP8 groups for the Eyes closed on foam test were also significantly higher than that for the OLDER UNEXPOSED group (p value range from Dunnett-Hsu test: 0.0022-0.025). However LOW group's response was significantly higher than that for OLDER UNEXPOSED group for pre log sway area variable for the Eyes closed on foam test only. A comparison of post-sway area of JP8 combined group (HI+MOD+LOW) with those of the YOUNGER UNEXPOSED group from our laboratory showed that JP8 group's responses for the EO, EC, FO and FC test conditions were 178%,130%, 275% and 202%, respectively higher than that for the unexposed group. The pre sway area responses for the JP8 group were 154%, 128%, 268% and 182%, respectively, higher than that for the YOUNGER, UNEXPOSED group.

Discussion/Conclusions:

The results are preliminary in nature, since the above analysis should be carried out with exposure variables other than naphthalene content of JP8. In our previous study with 30 air force maintenance personnel, we found that covariate adjusted regression analysis showed statistically significant increase in postural sway with an increase in chronic exposure to solvents contents (benzene, toluene and xylene) of JP8. While the present study appears to show some acute effects on postural balance, our previous studies with air force maintenance personnel indicated that low-level, long-term, chronic exposure to JP8 showed statistically significant increases in postural sway. The present study, since pre-sway values show significantly higher response than the unexposed groups (from our laboratory database), provides preliminary support to investigate further the chronic effects of JP8.

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Preliminary Report
Gene Environment Interactions and Exposure to JP8 Jet Fuel

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Introduction:

The characterization of genetic polymorphisms in genes that signal the production of key enzymes in the metabolism of components in jet fuel is being conducted to describe gene-environment interactions in personnel in the jet fuel study. Stratifying personnel by allele specific genotypes can help explain variability in health effects in personnel with similar levels of exposure, and to increase correlations between exposure to toxicants and a variety of measures of biological effect. Polymorphic enzymes may be more important at low-level exposures, since at high-level exposures, both high activity and low activity enzymes may be saturated.

Several of the genes involved in production of enzymes that activate and detoxify components in jet fuel are polymorphic. In the jet fuel study, allelic variants in three polymorphic genes, GSTT1, CYP2E1, and NQ01, are being characterized (genotyped). Relationships with health effects and other biological indicators of exposure are under investigation.

It has been reported that people vary in susceptibility to adverse health effects of chemicals such as benzene, a neurotoxic and hematotoxic component in jet fuel, partly because of interindividual variations in metabolic enzymes that activate and detoxify the toxicant (Wiencke et al, 1997, Snyder and Hedli, 1996). Metabolism plays a critical role in benzene toxicity. The polymorphic enzyme CYP2E1 converts benzene to benzene oxide, which is spontaneously rearranged to phenol. Phenol is oxidized via CYP2E1 to hydroquinone and other hydroxy metabolites, which are converted in the bone marrow by myeloperoxidase to genotoxic and hematotoxic benzoquinones. Polymorphic NAD(P)H quinone oxidoreductase (NQO1) catalyzes the conversion of benzoquinones to less reactive metabolites. In addition, benzene oxide is detoxified by conjugation to glutathione via the polymorphic glutathione S-transferase (GST). High CYP2E1 activity, low NQO1 activity and/or low GST activity may alter the production of toxic metabolites. This can alter interactions with critical macromolecules and affect toxic outcomes.

Approximately 50% of healthy people carry the C to T transition at base pair 609 of exon 6 in the NQO1 gene. There is a three-fold decrease in enzyme activity associated with this allele in the heterozygous state, and a total loss of activity in the homozygous state (Rothman et al, 1997, Wiencke et al, 1997). Approximately 30% of the US population does not produce the GSTT1 enzyme because of a homozygous deletion of the GSTT1 gene (Ketterer et al, 1992). The minor DraI allele C in the CYP2E1 gene is present in about 10-14% of the population and provides a high activity enzyme. Association of the CYP2E1 DraI allele C with DNA adducts was found to be greatest in individuals with low-level exposures (Kato et al, 1995).

Methods:

Assays to characterize CYP2E1, GSTT1, and NQO1 genotypes were determined following gene amplification using polymerase chain reaction (PCR) methodology. DNA was isolated at Texas Tech University. Genotyping was performed on DNA isolated from blood donated both before (pre) and after work (post). CYP2E1 Dra1 was identified by methods described by Kato et al, 1994. GSTT1 genotyping was performed by a slight modification to methods described in Pemble et al, 1994. NQO1 genotyping was performed according to methods described in Wiencke et al, 1997.

Chi-squared tests were used to determine if the proportion of genotypes varied across exposure categories. Linear models were used to determine if genotype interacted with exposure on endocrine, liver, and kidney function. Separate sets of models were used for exposure categories and for continuous measures of exposure.

Results:

Genotype analysis for CYP2E1, GSTT1, and NQO1 was completed for the 316 participants who provided blood for DNA preparation. Results using DNA isolated from pre and post blood had complete concordance. All 316 participants were characterized for the three genes.

CYP2E1 Genotype Analysis

The CYP2E1 DD genotype was found in 85.4 percent of participants. The CYP2E1 CD was found in 13.3 percent of participants. The CYP2E1 CC genotype was present in 1.3 percent of participants. Distribution of the CYP2E1 genotypes across the three exposure categories (low, moderate, high) is shown in Table 1. The frequency of genotypes did not vary significantly across the exposure categories (p=0.3546).

Table 1: Distribution of CYP2E1 Genotypes in Exposure Categories in 316 Participants

Genotype	Low	Moderate	High
CYP2E1 DD	132	40	98
CYP2E1 CD	22	3	17
CYP2E1 CC	1	0	3

GSTT1 Genotype Analysis

The deletion of the GSTT1 gene was found in 21.8 percent of 316 participants. The GSTT1 gene was present in 78.2 percent of participants. Distribution of the genotypes across the three exposure categories is shown in Table 2. The frequency of genotypes did not vary significantly across exposure categories (p=0.5985).

Table 2: Distribution of GSTT1 Genotypes in Exposure Categories in 316 Participants

Genotype	Low	Moderate	High
GSTT1 +	121	36	90
GST1 null	34	7	28

NQO1 Genotype Analysis

The NQO1 CC genotype was present in 64.6 percent of the 316 participants. The NQO1 CT genotype was present in 31.3 percent of participants. The NQO1 TT genotype was present in 4.1 percent of the participants. Distribution of the NQO1 genotypes across the three exposure categories is shown in Table 3. The frequency of genotypes did not vary significantly across exposure categories (p=0.4751).

Table 3: Distribution of NQO1 Genotypes in Exposure Categories in 316 Participants

Genotype	Low	Moderate	High
NQO1 CC	104	29	71
NQO1 CT	47	11	41
NQO1 TT	4	3	6

Analysis of Gene Environment Interactions

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotypes interacted with exposure and a-GST (n=292). Alpha-GST in serum is an indicator of early hepatic damage. There were no statistically significant effects indicating an interaction of genotype with exposure in the 292 participants.

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotype interacted with exposure and urinary a-GST (n=304). Urinary a-GST is an indicator of early damage to the proximal tubules in the kidney. There were no statistically significant effects indicating an interaction of genotype with exposure in the 304 participants.

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotype interacted with exposure and urinary pi-GST (n=307). Urinary pi-GST is an indicator of early damage to the distal tubules in the kidney. There were no statistically significant effects indicating an interaction of genotype with exposure in the 307 participants.

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotype interacted with exposure and endocrine function measured by serum endocrine concentrations in 130 men. Interactions were examined with levels of serum luteinizing hormone, follicle stimulating hormone, prolactin, free and total testosterone, estradiol, inhibin B, and cortisol. For endocrine function, there were no statistically significant effects indicating an interaction of genotype and exposure.

Summary/Conclusions:

Final analysis of gene-environment interactions in the JP8 jet fuel study will be completed as soon as data are available on all outcome measures. Analysis will be simplified because results of the genotype analysis in the 316 participants have shown that there is no significant difference in distribution of genotypes across exposure categories. Exposure will be refined by including measures of dermal exposure and blood levels of JP8 jet fuel components. Gene environment interactions will be examined in the postural sway test, an assessment of a

participant's control mechanism for governing balance and in the conditional eyeblink response, which can identify cognitive deficits. In addition, gene interactions will be evaluated in the GASH/BARS system that is designed to measure motivation, response speed, coordination, grip strength, complex mental functioning, memory, and attention. Furthermore, interactions with passive benzene and naphthalene exposure, exhaled and urinary benzene and naphthalene, and molecular adducts of naphthalene may be described.

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Final Report
Sensitive early indicators of hepatic and kidney damage in workers exposed to Jet Fuel
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Sensitive assays for liver and kidney damage provide an opportunity to evaluate early hepatic and renal damage in workers exposed to JP8 jet fuel. In this study, an assay for the cytoplasmic enzyme, plasma alpha-glutathione S-transeferase (alpha-GST), tested the association between JP8 exposure and hepatic damage. Over 85% of alpha-GST in humans is found in hepatocytes and is released into the blood upon hepatic damage. As a result of its short half-life, plasma alpha-GST rises rapidly when hepatocytes are damaged and returns rapidly to baseline when damage is repaired. Plasma alpha-GST is a more sensitive indicator of damage than alanine aminotransferase and aspartate aminotransferase (Clarke et al, 1997; Rees et al, 1995). In an earlier study, JP8 workers had no significant changes in liver function measured by alanine aminotransferase and aspartate aminotransferase. However, some have questioned the use of these measurements for assessing hepatocyte damage since levels may be normal in some people with chronic liver disease (Clarke et al, 1997).

Two additional tests which measure urinary alpha or pi glutathione S-transferase (GST) are more sensitive for detection of early kidney damage than serum creatinine and blood urea nitrogen assays. The latter assays require a considerable loss of renal capacity before test values are significantly elevated. In earlier work, there were no significant differences in kidney function measured by BUN and creatinine in JP8 exposed and unexposed workers(Gould, 1998). . In the kidney, alpha-GST is located only in the epithelial cells in the proximal tubule; pi-GST is localized within the epithelial cells in the distal tubules (Sundberg et al, 1994). Monitoring the urinary levels of these enzymes has proven valuable in monitoring kidney transplant patients to differentiate between transplant rejection and nephrotoxicity without the necessity of a biopsy (Sundberg et al, 1994).

For this research, enzyme-immunoassays were used to measure hep alpha-GST in subject plasma to monitor early signs of hepatic damage; neph alpha- and pi-GST in urine were used for the detection of early kidney tubule damage.

Methods:

Study population: Exposed workers were tank-entry personnel with at least nine months of persistent exposure to jet fuel, i.e., one-hour entry, twice a week, validated against shop records. The unexposed group consisted of US Air Force personnel who do not routinely work with or have significant exposure to fuels or solvents. Participants were chosen from among those meeting eligible criteria from six USAF bases: Davis Monthan AFB, AZ, Seymour Johnson AFB, NC, Langley AFB, VA, Pope AFB, NC, Little Rock AFB, AR, and Hurlbert Field, FL.. Exclusion criteria are history of autoimmune disease, cancer, diabetes, and immune altering medication. Participants received $50 for their time and inconvenience.

Participants completed a questionnaire to provide job, exposure, medical, and demographic information.

Sample Collection: Venous blood samples were collected from 107 individuals using 10 ml EDTA tubes. Pre- and post-shift samples were collected for all subjects. Blood samples were shipped on ice to the NIOSH laboratory by next day courier. At the NIOSH laboratories, samples were spun in a centrifuge to separate plasma from packed cells and plasma was transferred to screw-top polypropylene cryovials (Cat no. 60-542, Sarstedt, Inc.) approximately 24 hours after blood collection, and kept frozen at –80°C until assayed. Pre- and post-shift urine samples were collected from subjects and shipped on ice to the NIOSH laboratory by next day courier. At the NIOSH laboratories samples were divided into aliquots for respective studies. Samples destined for determination of neph alpha- and pi-GST were prepared for storage by the addition of stabilizing buffer (Biotrin International, 20% urine volume) and samples were frozen at –80° C until thawed for assay.

All samples were randomly numbered for blinded analysis. Plasma samples were thawed and assayed in duplicate for Hep alpha GST using commercial immunoassay kits (Biotrin International, Cat # BIO60HEPA). Urine samples were thawed and assayed for both neph alpha- and pi-GST using commercial immunoassay kits (Biotrin International, BIO66NEPHA and BIO69NEPHPI, respectively).

Statistical Analyses:

Pearson correlation coefficients were derived for each of the enzyme variables as well as pre- and post-shift change against the following variables:

Age, Base, Mthjob, Hisp, Pexert, Mental, Natlogpass, Natlogpre_n, Natlogpost_n, height, weight, BMI, Smoker, Alcohol, Alcdown, Alcbout, Alcuse, Alcsitng, Physwrk, Physntwk, Analyco24, and Noalcdurstudy.

Univariate analyses of variance were conducted for each enzyme variable against the following variables:

Race, Hisp, Category, Smoker, Alcohol, Analyco24, and Noalcdurstudy.

Status:

Analysis for hep alpha-GST for plasma and neph alpha- and pi-GST in urine samples from all subjects have been completed and statistical analysis conducted as described above.

Findings:

Assessment of liver toxicity; Levels of serum hep alpha-GST in the study subjects were well within the normal range for this measure; no differences were observed indicative of hepatic changes attributable to any of the variables examined (Table 1).

Assessment of kidney toxicity; Levels of urinary neph alpha- and pi-GST in the study subjects fell within the normal range for healthy subjects; no differences were observed indicative of renal changes attributable to any of the variables examined (Table2) .

Assessment of urine creatinine; Creatinine was used to normalize neph alpha- and pi-GST to correct for urinary dilution. One finding of this study was that individuals from exposure category 3 had significantly higher levels of urinary creatinine in their post shift samples. While the mean values are within normal ranges (0.25-4.0 mg/ml) for this measure in healthy

individuals and are not indicative of clinical disease, they are evidence of more concentrated urine (Table 3).

Discussion/Conclusions:

The study group represents a very healthy segment of the population. Sensitive measures for liver and kidney damage did not detect any adverse effects in specimens from this study group. Evidence of elevated creatinine in the mean post-shift samples of exposure category 3 was seen. However, while these values are within normal clinical ranges, they are consistent with concentrated urine indicative of mild dehydration. Because of the elevated urine creatinine levels in post-shift samples of exposure category 3, there is an apparent decrease in pre- vs. post-shift levels of Neph alpha- and pi-GST in these samples. This is an artifact of the concentrated creatinine. When levels of urinary GSTs are expressed on a per volume basis, levels remain unchanged between pre- and post-shift samples.

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Table 1. Effect of JP8 Exposure Category on Hepatic Alpha Glutathione Transferase (Hep Alpha GST).

Exposure Category	Hep Alpha GST (µg/L)	Hep-Alpha GST Normal Range (µg/L)	Hep-Alpha GST Pre-Post Difference (µg/L)
1 Pre	2.82 ± 2.46	0-11	-------
2 Pre	3.01 ± 2.32	0-11	-------
3 Pre	2.73 ± 2.34	0-11	-------
1 Post	2.81 ± 4.94	0-11	-0.01
2 Post	2.96 ± 2.08	0-11	-0.05
3 Post	2.67 ± 2.50	0-11	-0.06

Table 2. Effect of JP8 Exposure Category on Kidney Proximal Tubule Glutathione Transferase (Neph Alpha GST) and Kidney Distal Tubule Glutathione Transferase (Neph Pi GST).

Exposure Category	Neph-Alpha GST (μg/mg creatinine)	Neph-Alpha GST Normal Range (μg/mg creatinine)	Neph-Alpha GST Pre-Post Difference (μg/mg creatinine)	Neph-Pi GST (μg/mg creatinine)	Neph-Pi GST Normal Range (μg/mg creatinine)	Neph-Pi GST Pre-Post Difference (μg/mg creatinine)
1 Pre	2.13 ± 5.03	0-10	-------	4.71 ± 5.61	0-12	-------
2 Pre	1.43 ± 2.86	0-10	-------	5.00 ± 5.12	0-12	-------
3 Pre	1.02 ± 2.39	0-10	-------	4.32 ± 4.95	0-12	-------
1 Post	2.44 ± 5.74	0-10	0.31	5.71 ± 7.65	0-12	+1.0
2 Post	1.46 ± 2.67	0-10	0.03	5.07 ± 8.05	0-12	+0.07
3 Post	0.71 ± 2.83	0-10	-0.29	2.93 ± 3.20	0-12	-1.39

Table 3. Effect of JP8 Exposure Category on Urine Creatinine Levels.

Exposure Category	Urine Creatine (mg/ml)	Urine Creatinine Normal Range (mg/ml)	Urine Creatine Pre-Post Difference (mg/ml)
1 Pre	1.82 ± 1.01	0.25-4.0	-------
2 Pre	1.71 ± 0.96	0.25-4.0	-------
3 Pre	1.96 ± 0.96	0.25-4.0	-------
1 Post	1.62 ± 0.90	0.25-4.0	-0.20
2 Post	1.84 ± 0.97	0.25-4.0	+0.13
3 Post	2.54 ± 1.15	0.25-4.0	+0.58

The human glutathione-S-transferase M1 (GSTM1) polymorphism as a risk factor for acute toxicity from jet fuel exposure

Introduction:

Within the human population, there is often a wide range of possible responses to a single toxic exposure. Genetics, lifestyle choices, age, disease, ethnicity, and exposure history all play a role in this differential susceptibility. In this study, we explore the role of a specific xenobiotic-metabolizing enzyme, glutathione-S-transferase M1, in risk for developing acute toxicity from jet fuel exposure. GST M1 is important for this work because, as a detoxification enzyme, it shows protective effects against cellular damage in a number of test systems. It is also important because approximately 50% of the US population do not express this enzyme. Several case-control studies suggest that the GST M1-null genotype is over-represented in patients with certain types of cancer. It has also been reported that the GST M1-null genotype is more prevalent among patients with neuropsychiatric disabilities linked to organic solvent exposure. At the present time, however, there is no information regarding the role of GST M1 status in risk for adverse human health effects associated with JP8 exposure.

It is anticipated that, as part of the larger JP8 study, any acute health effects linked to exposure will be documented. The goal of our specific subproject is to determine whether GST M1 status is a possible *risk modifier* for any of these effects.

Methods:

From each volunteer in the study, we obtained blood samples before and after the work shift. From the blood, we isolated genomic DNA, which was used to determine whether an individual carried the gene for GST M1. A well-established polymerase-chain reaction (PCR) method was used to visualize amplification of a 273 base pair product of the gene. DNA templates that yielded no GST M1-specific PCR provided a means to identify all GST M1-null individuals. From the blood, we also isolated lymphocytes, which were used to measure GST mu-specific activity. With exposure to toxic agents, there is often concordant regulation of xenobiotic-metabolizing enzymes in the lymphocytes and liver or other tissues. However, in other cases, inducing agents may alter expression only in certain tissues. We wanted to determine, therefore, whether lymphocytes might act as a biomarker for metabolic changes thought to occur in other tissues. Activity was measured as the rate of an enzyme-mediated reaction conjugating tritiated trans-stilbene oxide (^{3}H-TSO) to glutathione. This reaction produces tritiated water, which can counted in the isolated aqueous phase after extraction with hexyl alcohol. In a standardized reaction, the higher the radioactivity, the more enzyme activity is present per mg of lymphocyte protein. TSO-conjugation activity was measured in all GST M1-positive individuals, identified beforehand by genotyping. GST M1 was also measured in a representative sample of GST M1-null individuals, which established a baseline cut-off value between GST M1-null and GST M1-positive individuals.

Status:

We have completed processing of samples, and have determined GST M1 genotypes for all study volunteers. We have also completed the determination of GST M1 activity (for phenotyping). We are in the process of determining GST 'total activity' in a subset of the population, though there is limited tissue availability for a complete analysis. Preliminary statistical analyses are presented

below in an effort to characterize the dataset, and compare our results with other published studies. For the purpose of risk assessment, final data analysis will be coordinated with statisticians on the project. At this time, integration with "effects data" has not yet begun. Therefore, we cannot determine whether or not GST M1 qualifies as a risk modifier of JP8 health effects.

Findings:

Gentotyping:

A total of 326 individuals were genotyped to determine the presence of at least one copy of the GST M1 allele. In the preliminary phase of this study, our research team:

- Determined that 164 individuals are GSTM1-null and 162 individuals are GSTM1-positive, for an overall incidence of 50.3 % GSTM1 null.
- Characterized significant differences in GST-mu genotype distribution based on race. For instance, of self-reported Caucasians, 53.3 % were GST M1-null (n = 246). Of self-reported African-Americans, only 30.0 % were GST M1-null (n = 40), a difference that was significant (p = 0.01). These differences are consistent with data from numerous other research groups. Individuals with mixed racial heritage or other minority races could not be statistically analyzed, due to low group sizes.
- Determined a significant positive trend between JP8 exposure category and incidence of the glutathione-S-transferase M1 (GSTM1) null genotype (p = 0.01 in chi-square test for trend). "Exposure" in this case was deduced from job titles and self-reporting of JP8 exposure. This overall positive trend was maintained when Caucasians and African-Americans were analyzed separately. Between the two lowest and two highest categories of JP8 exposure among Caucasians, the GST M1-null genotype increased from 45.4 % (n = 119) to 60.6 % (n = 127) a difference that was also significant (p = 0.02 in Fisher's exact test). For African-Americans, the GST M1-null genotype increased from 25.0 % (n = 24) to 37.5 % (n = 16). However, this difference was not significant, possibly due to low group numbers.
- Determined that the increased relative proportion of GST-null genotypes in the JP8 exposed group does not correlate with length of service.
- Determined no clear relationship between naphthalene concentrations in expired air at the end of the work shift and relative proportion of GSTM1 null genotype.

Phenotyping

For phenotyping, GST-mu activity was measured in lymphocytes from morning and evening blood samples in individuals determined to be GST-mu positive. Activity was also measured in a representative sample of GST-mu null individuals to establish background levels. In preliminary analyses, we:

- Determined a significant difference in lymphocyte GSTM1 activity between the GSTM1-null population and the GSTM1-positive population, as anticipated.
- Characterized large interindividual variability of lymphocyte GST-mu enzymatic activity among military personnel that express the allele(s) for GST M1.
- Determined that there is no clear association between lymphocyte GST-mu activity and JP8 exposure, using a variety of exposure criteria.

In the next month, there will be further characterization of the dataset. At this time, all demographic variables have not been addressed. In the final phases of this study, we will be interacting with other investigators to determine whether differences in GST-mu genotype and phenotype have any bearing on risk for acute toxicity from occupational exposure to JP8.

Discussion/Conclusions:

The increase in relative proportions of the GST-mu null genotype as a function of exposure category was not expected. However, there appears to be some precedent in the literature. GST-mu nulls are over-represented among smokers and in some cancer patient populations. In our study, "selection" on the basis of genotype does not appear to be a consequence of JP8 exposure per se, since length of service was not a significant factor. Moreover, relative proportions of each genotype showed no relationship to "high" and "low" concentrations of naphthalene in breath from workers that were acutely exposed to JP8. Therefore, there may be other genetic, social, or environmental links to partially explain the observed trend. For instance, there were more smokers in the "high exposure" category compared to the "low exposure" category. This study therefore supports other published work indicating that smokers are more likely to have the GST M1-null genotype. Although mechanisms for the smoker-GST M1 link are not clear, it has been suggested that GST M1-null individuals are more susceptible to nicotine-dependence. However, further statistical analyses will need to be performed to determine whether smoking status alone can explain the positive trend between the relative proportion of GST M1-nulls in the population and JP8 exposure.

In our dataset, GST M1 was expressed in approximately 53.3 % of Caucasians. This is similar to published reports. By contrast, African-Americans showed a significantly lower percentage of GST M1-nulls, 25 %, which is also similar to published reports. Other racial/ethnic differences could not be established in this dataset, due to low numbers. It is clear that more research is required to characterize GST M1 genotype distributions in these minority populations.

Our failure to show a link between JP8 exposure and lymphocyte GST-mu activity does not rule out GST-mu specific responses in other tissues, and it does not mean that other lymphocyte GST isoforms are not modulated by JP8 exposure. Tissue-specific regulation is a common characteristic of many xenobiotic-metabolizing enzymes (XMEs), including GST M1. Animal studies, for instance, show that GST-mu is expressed in several specific areas of the brain, and expression is increased after exposure to JP8. However, our data suggests that changes in lymphocyte GST-mu activity probably do not represent a reliable biomarker for JP8 exposure in humans, at least at levels commonly encountered on military bases. If there were changes in lymphocyte expression of this enzyme, high interindividual variability would tend to obscure the significance. It remains to be determined whether GST-mu activity in lymphocytes is a reliable biomarker of effect, although wide interindividual variability will likely continue to be an issue in this case.

As effects data are compiled, we will analyze for any difference between GST M1-nulls versus GST M1-positive individuals in the various exposure categories. Significant differences, based on genotype, could imply a GST M1-mediated mechanism of resistance to toxicity. As others have suggested, however, interindividual differences in metabolism may only be important in the low-to-moderate range of exposure. At very high levels of exposure to toxic compounds, adaptive and detoxification strategies tend to be overwhelmed, and interindividual differences in susceptibility are

thus obliterated. Other investigators may need to take this toxicological principle into account. It should also be taken into account that GST M1-related differences may be modified by still other genetic and lifestyle factors. We will be working closely with Mary Ann Butler at NIOSH to determine if there are any gene-gene interactions that could add or subtract from identified risk.

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The Effects of JP8 Jet Fuel on Serum Endocrine Concentrations in Men: Risk Assessment of Acute Exposure to Jet Fuel

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Introduction:

Evidence from animal and human studies suggest that components of JP8 jet fuel can disrupt the neuroendocrine axes that control or impact the reproductive and immunologic systems [1-12]. Human studies have demonstrated reduced secretion of luteinizing hormone (LH) associated with toluene and JP8 exposure [9-12]. LH is critical for reproductive performance, but also effects immunologic functions by increasing interleukins involved in natural killer cell activation and B-cell differentiation [1]. Adrenocorticopic hormone, follicle stimulating hormone (FSH), prolactin, corticosterone, testosterone, and estradiol also regulate immune function and are affected by compounds in fuels and exhaust [1-12].

JP8 represents the most common chemical exposure among members of the Armed Forces. The specific objective of this aspect of the study is to **determine endocrine levels in the peripheral blood and whether these endocrine concentrations are associated with JP8 exposure or other work conditions. Information gathered in this study would help the National Institute for Occupational Safety and Health (NIOSH) establish recommended exposure limits for military and civilian aviation.**

Methods:

Exposed workers were male tank-entry personnel with at least nine months of persistent exposure to jet fuel, i.e., one-hour entry, twice a week, validated against shop records. The unexposed group consisted of US Air Force personnel who do not routinely work with or have significant exposure to fuels or solvents. Participants were chosen from among those meeting eligible criteria from three USAF bases: Pope, Little, and Hurlburt. Exclusion criteria are history of autoimmune disease, cancer, diabetes, and immune altering medication. Participants received $50 for their time and inconvenience.

Participants completed a questionnaire to provide job, exposure, medical, and demographic information. In addition, venous blood samples were collected from 153 individuals using 10 ml Serum Separation Tubes with clot activator and twice the polymer barrier (Cat no. 367985, Becton, Dickinson and Co.). Samples were collected in the morning at about the same time for all subjects, to minimize the confoundment of diurnal rhythms. Blood samples were inverted 5 times and allowed to set for 30-60 minutes at room temperature to allow the blood to clot before centrifugation at 1,000-1,300 g for 10 minutes. Separated samples were immediately refrigerated and shipped on ice to the NIOSH laboratory by next day courier.

At the NIOSH laboratories, serum was transferred to screw-top polypropylene cryovials vials (Cat no. 60-542, Sarstedt, Inc.) approximately 24 hours after blood collection, and kept frozen at -80°C until assayed.

Serum samples were randomly numbered for blinded analysis. Serum samples were assayed in duplicate for each hormone; inhibin-B duplicates were drawn from a single pre-treatment aliquot. Quality control serum pools (2-3 levels) were assayed at the beginning and end of each assay.

FSH & LH were measured using DELFIA noncompetitive, microtiter immunofluorometric assays (cat. no. A017-201 & A031-101, respectively; PerkinElmer-Wallac). Inhibin-B was measured using a noncompetitive, microtiter enzyme immunoassay (cat. no. MCA1312KZZ; Serotec, Inc); inhibin-B values were adjusted to correct for a slight shift across the microtiter plate. Prolactin was measured using a tube-based noncompetitive immunoradiometric assay (cat. no. DSL-4500; Diagnostic Systems Laboratories, Inc (DSL). Cortisol was measured using a coated tube, competitive radioimmunoassay (cat. no. DSL-2100; DSL). Estradiol was measured using a sensitive, double antibody, competitive, ultra-sensitive radioimmunoassay (cat. no. DSL-4800; DSL). Total and free testosterone were measured using coated tube, competitive radioimmunoassays (cat. no. TKTT & TKTF, respectively; Diagnostic Products Corp.).

Of the 153 individuals who provided morning blood samples, 17 were excluded who were women and 2 were excluded who provided only afternoon blood samples.

Pearson correlation coefficients were derived for each of the 8 hormone endpoints against the following variables: *Age, Base, Mthjob, Hisp, Pexert, Mental, Natlogpass, Natlogpre_n, Natlogpost_n, height, weight, BMI, Smoker, Alcohol, Alcdown, Alcbout, Alcuse, Alcsitng, Physwrk, Physntwk, Analyco24, and Noalcdurstudy.*

In addition, linear models were used to assess the effect of exposure (*Cat2*) and months-on-the-job (*MthJob*) and their interaction (*Cat2* x *MthJob*), while controlling for age, smoking, and alcohol-use. MthJob, age, smoking, and alcohol-use were continuous variables, exposure was a classification variable. A separate model was conducted for each hormone.

Status:

All venous blood samples collected for this aspect of the study have been analyzed for endocrine concentrations. Statistical analyses have been conducted as described herein.

Findings:
Correlations that statistical significance at the P < 0.01 level:

Correlates Variable Vs. Endpoint	Correlation Coefficient	P-Value
MthJob vs. Total	r = - 0.263	P = 0.002
MthJob vs. Free	r = - 0.278	P = 0.001
Age vs. FSH	r = 0.347	P =
Age vs. Free Testosterone	r = - 0.263	P = 0.002
Smoker vs Prolactin	r = 0.227	P = 0.009
Alcdown vs Total	r = - 0.350	P <
Alcbout vs Total	r = - 0.272	P = 0.002
Alcsitng vs Total	r = - 0.254	P = 0.004

For FSH, the main effect of exposure was significant (p = 0.03), though none of the adjusted means were significantly different from each other. The Cat2 x MthJob interaction approached significance (p = 0.06): while the slopes describing the relationship between months-on-the-job and low (b = 0.0019, p = 0.7) and moderate (b = -0.0086, p = 0.2) exposure groups were not different from zero, the slope for the high exposure group tended to be greater than zero (b = 0.015, p = 0.055). FSH level was also directly related to age (b = 0.10, p = 0.005).

The main effect of exposure was significantly (p = 0.035) related to inhibin B levels. Adjusted serum levels of the high exposure group (205 mIU/ml) were significantly greater than that for the moderate exposure group (167 mIU/ml).

Increased smoking was significantly related to reduced prolactin levels (b = 0.59, p = 0.013) and tended to be associated with reduced total testosterone levels (b = 0.23, p = 0.059). Estradiol levels decreased with increased alcohol use (b = 0.21, p = 0.028).

There was no indication that naphthalene exposure or month-on-the-job affect serum levels of testosterone, estradiol, LH, prolactin, or cortisol. There were no significant main effects or interactions for LH, cortisol, or free testosterone.

Endocrine Endpoint	Effect	P-Value	Description
FSH	Cat2	p = 0.03	Adjusted means are not different.
	Cat2 x	p = 0.06	Only MthJob x High Exp slope tended to differ
	Age	p =	FSH may increase with age (b=0.10).
Inhibin B	Cat2	p =	Inhibin B levels may be higher for High Exp
Prolactin	Smoking	p =	Prolactin may decrease with smoking
Total	Smoking	p =	TT tends to decrease with smoking (b=0.23).
Estradiol	Alcohol	p =	Estradiol may decrease with alcohol use

Discussion/Conclusions:

These preliminary statistical analyses reveal statistical trends suggesting that FSH levels may be higher in AF personnel who have worked for longer duration in jobs with higher naphthalene exposure. These results also suggest that men with high naphthalene exposure experience elevated inhibin B levels. Inasmuch as inhibin B exerts negative feedback on FSH secretion, this scenario is consistent with an exposure effect either stimulating FSH secretion leading to elevated inhibin B levels, and/or a relative desensitization of the feedback setting.

Month-on-the-job was also inversely correlated with testosterone levels. This association, however, disappeared upon including age in the multivariate model.

Preliminary analyses would suggest that heavy smokers might experience reduced prolactin and testosterone levels. Preliminary analyses reveals that the heavy alcohol consumption is associated with reduced prolactin levels. There wis also a direct correlation between the amount of alcohol consumed and testosterone levels, however this relationship was not apparent with multivariate analyses.

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The Effects of Heat Stress on Air Force Employees Conducting Fuel Cell Maintenance Activities on Air Force Jets

Introduction:

The degree to which metabolic responses occur in reaction to hot environments differs per individual; however, when any human body is exposed to heat and the internal (core) body temperature rises, the body must rid itself of the excess heat. It does this automatically by increasing cardiac output and expanding larger blood vessels to accommodate the increased flow.[1] This heat-stress-induced increase in the body's metabolism is potentially a confounding variable in the association between jet fuel constituent metabolism and performance and health measures.

Of the 324 persons who completed the study, a total of 140 employees, not including those at Dyess AFB, were monitored for core body temperature as well as other measures of metabolic activity including skin and ear temperatures, heart rate, and gross motor activity.

These measurements of heat stress and heat strain are being compared to some of the many heat stress guidelines that have been developed to protect people against heat-related illnesses. The objective of any heat stress index is to prevent a person's core body temperature from rising excessively; the World Health Organization concluded, "It is inadvisable for deep body (core) temperature to exceed 38 °C (100.4 °F) in prolonged daily exposure to heavy work."[2] NIOSH guidelines also use a maximum core body temperature of 38 °C as the basis for their environmental criteria.[3] The American Conference of Governmental Industrial Hygienists (ACGIH) offers additional physiological guidelines, as well. For individuals with normal cardiac performance, sustained heart rate should not exceed 180 beats per minute *minus age*; the core temperature of unacclimatized workers should not exceed 38 °C (100.4 °F), while the core temperature of those workers who are accustomed to the work environment (acclimatized) should not exceed 38.5 °C (101.3 °F). Finally, a worker should not experience profuse and prolonged sweating or symptoms of sudden and severe fatigue, nausea, dizziness, or lightheadedness, or lose more than 1.5% of body weight over the shift.[4]

Methods:

With the development of new technology, measuring core body temperature has only very recently become a viable option for research and industrial applications. During this study, NIOSH researchers used the CorTemp Wireless Core Body Temperature Monitoring System™ to monitor up to six employees daily. The CorTemp Temperature Sensor is swallowed and provides continuous monitoring of core body temperatures until the sensor is passed from the body, about 72 hours after being swallowed. The sensor has a temperature-sensitive crystal that vibrates in direct proportion to the temperature of the surrounding body tissues. This vibration creates an electromagnetic flux that continuously transmits harmlessly through the body tissues. A recorder receives this signal and translates it into digital temperature information that is then displayed on the unit and simultaneously stored to memory.

Heart rate, gross motor activity, skin temperature, and ear temperature monitoring were also conducted using the Mini-Mitter Mini-Logger Series 2000®. Employees wore an aural (ear)

temperature probe, a skin temperature probe, Polar chest-band heart rate monitor, and an activity sensor on the wrist.

Environmental factors most nearly correlated with core body temperature and other physiological responses to heat were also monitored using two wet bulb globe temperature (WBGT) instruments. One was placed inside the hangar with the fuel cell maintenance employees, and a second was placed outside to monitor outdoor environmental conditions every day during the study.

Status:

Currently, analyses are being conducted on the environmental and physiological measurements that were collected in order to assess the extent of heat stress and strain experienced by each employee. Analyses will also help identify variables that will in turn help other study researchers identify the potential and extent of heat stress confounding.

Findings:

Analyses are currently ongoing.

Discussion/Conclusions:

Site-specific NIOSH reports will be completed and forwarded to management and employee representatives at each Air Force base included in the study. These reports will include sampling results for groups of employees only (no individual data will be provided), and will provide recommendations for abating heat stress conditions and heat strain, if applicable.

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The Effects of JP8 Jet Fuel on Immune Cell Counts of Tank Entry Workers

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Abstract

Jet fuel is a common occupational exposure among commercial and military maintenance workers. JP8 jet fuel, a military formulation, has been found to have immunotoxic effects in mice but little data exists for humans. The aim of this cross-sectional study was to determine if immune cell counts in the peripheral blood were altered among tank entry workers at three Air Force bases. After adjusting for covariates, tank entry workers (n=45) were found to have higher numbers of white blood cells (p=0.01), neutrophils (p=0.05), and monocytes (p=0.02) when compared to a low exposure group (n=78) and no differences were noted in the numbers of total lymphocytes, T-cells, T-helper cells, T-suppressor cells, Natural Killer cells, and B-cells. Further investigations are under way to evaluate the functional ability of these cells to produce lymphokines and cytokines and modulate the immune system.

Introduction

The worldwide consumption of jet fuel approaches 60 billion gallons annually.[1] According to the U.S. Department of Labor, Bureau of Labor Statistics, 1.3 million workers were exposed to jet fuels in 1992.[2] The U.S. Department of Defense uses Jet Propellant fuel type eight (JP8), one formulation of jet fuel, at a rate of 3.5 billion gallons yearly, of which the Air Force is the largest consumer. JP8 is the battlefield fuel for all U.S. military operations and is expected to be in use well beyond the year 2025.[3]

JP8 is a kerosene-based fuel similar to commercial aviation fuel Jet A and Jet A-1, but has military additives which include antioxidants, static inhibitors, corrosion inhibitors, fuel system icing inhibitors, and thermal stability improvers.[4] In 1996, JP8 replaced JP-4, which was a more volatile and explosive, gasoline based fuel. JP8 contains benzene, a proven carcinogen, but contains less per volume than JP-4 (0.01% versus 0.5%).[5,6]

In the Air Force, persons having the highest exposure to JP8 are tank entry personnel.[5] These persons enter aircraft on-board fuel tanks to perform inspections and maintenance activities. They are exposed to residual fuel in the tanks and to fuel released from reticulated polyurethane foam. The foam is fitted in fuel tanks of various aircraft and serves to reduce the risk of explosion from electrical arcing, lightening strikes, and static electricity. Fuel tanks with foam are less likely to explode if struck by ballistics or involved in a crash as the foam prevents fuel sloshing and spraying in the event of tank rupture. Tank entry personnel work in groups of three. The entrant works within the confined space of the tank and wears a respirator. Two attendants work near the tank entry port fetching tools and handling the fuel impregnated foam, which is usually stacked on the aircraft wing while maintenance is occurring within the tank. The two exterior attendants do not wear respirators and therefore may have inhalation exposure to jet fuel while working near the tank opening or handling foam. All three wear cotton clothing rather than impermeable garments, which may generate static electricity. Fuel left within tanks or released from foam during handling, may be readily absorbed and deposited onto the skin creating a dermal exposure.

While the Occupational Safety and Health Administration (OSHA) has not developed a permissible exposure limit (PEL) for jet fuel, the Air Force has occupational exposure limits of 350 mg/m^3, Time Weighted Average (TWA) over 8 hours and 1,800 mg/m^3 for short term exposures over 15 minutes. Tank entry personnel handling foam have been found to have exposures as high as 1,304 mg/m^3 for an 8-hour TWA and 10,295 mg/m^3 for a 15 min short-term exposure.[5]

After the introduction of JP8, fuel handlers complained of objectionable odors, skin irritation, dizziness and the persistent taste of jet fuel long after exposure.[7] Health outcome studies in humans of the effect of JP8 are limited, but some effects have been reported. Genotoxic changes as evidenced by sister chromatid exchanges were noted in aircraft maintenance workers. However, no male reproductive effects have been noted on male semen parameters.[8] Neurological disorders and hearing loss were also noted in aircraft maintenance workers occupationally exposed to jet fuel.[9] Other reports on Swedish workers exposed to jet fuel cite differences in psychiatric symptoms, attention, and sensorimotor effects when compared

to nonexposed workers.[10,11,12] Postural balance deficiencies were noted in workers exposed to chronic low-levels of jet fuel over an average of 4.6 years.[13]

A reference report, published in 1998, by the Center for Disease Control's Agency for Toxic Substances and Disease Registry, indicated the toxicities of jet fuel are not well defined.[14] The immune system was identified as one area where human health effects needed further study and was the purpose of this research project.

Background

The immune system is responsible for regulatory responses to infection, cancer, autoimmune disease, and allergens. The spleen, thymus, lymph nodes, bone marrow, blood and other organs have cells involved in the immune response. In the peripheral blood, immune cells are represented by white blood cells that consist of lymphocytes, neutrophils, basophils, eosinophils, and monocytes.

Lymphocytes have several subpopulations that can be delineated by cluster designation (CD). T-cells (CD3) and B-cells (CD19) orchestrate the entire immune response.[15] T-cells consist of T-helper cells (CD4) and T-suppressor cells (CD8) that modulate cell-mediated immunity both directly and by the secretion of lymphokines and cytokines. Natural Killer (NK) cells (CD56) target cancer cells.

Most immune system toxicology studies concerning the health effects of jet fuel have been undertaken in mice. Exposure to inhaled benzene (a component of jet fuel) at levels of 50 ppm to 200 ppm over 7 days and 14 days produced a decreased ratio and absolute number of T-cells and B-cells in the blood and spleen.[16] The effect was dose dependent and resulted in a suppressed ability to form antibodies. Subpopulations of T-cells were not addressed in the above benzene study and jet fuel as a complex mixture was not evaluated. However, the level of benzene exposure in the above study would be comparable to the levels (104 ppm to 142 ppm) found in Air Force workers exposed to JP8.[6]

Short-term exposure of mice to JP8, by inhalation one hour daily for seven days at levels comparable to tank entry worker exposure, produced a dose response decrease in weights of the spleen and thymus, and a reduction in T-cell subpopulations in the lymph nodes.[2] A decrease in circulating immune cells at low (100-250 mg/m^3) concentrations was noted whereas at medium (500-1,000 mg/m^3) concentrations the number of cells increased. High (2,500 mg/m^3) concentrations appeared to be toxic to peripheral blood immune cells. Total T-cells were noted to decrease significantly at doses as low as 250 mg/m^3 in the peripheral blood but absolute number and ratio of T-helper and T-suppressor cells were not evaluated. Macrophage percentages were also noted to decrease by two-thirds at low and high concentrations compared to unexposed mice. The long-term effects of the same short-term exposure noted above were studied in mice until 28 days post-exposure.[17] The weights of the spleen and thymus initially decreased, returned to normal, and finally increased. At the high exposure of 2,500 mg/m^3, immune cell numbers in the peripheral blood were substantially decreased at 1, 7 and 21 days, but were not noted to be statistically different from unexposed controls at 14 and 28 days. Again the subpopulations of immune cells were not delineated.

In experiments with mice exposed to JP8 by dermal absorption, impairment in the induction of contact sensitivity and the generation of delayed-type hypersensitivity were found when the mice were challenged several days later by antigens.[18] Splenic T-cells were noted to have significantly decreased proliferation rates compared to positive controls when stimulated, indicating a reduction in the functional capacity of the immune system. The number of circulating immune cells in the peripheral blood was not determined.

In humans, few immunotoxicity studies have been reported on exposure to jet fuel. One of note was a pilot study of exposed and unexposed workers, during the conversion of JP-4 to JP8.[6] In this study by Olsen, et al, differences in the hematopoietic system were noted but no significant findings were found in the immune system. Mean corpuscular hemoglobin and mean corpuscular volume were significantly lower in the exposed group while immune cells (total white blood cell counts and differential counts) were not significantly different. The sample size was small however, (18 exposed and 18 unexposed) and the lymphocyte subpopulations were not studied.

Methods

This investigation was a cross-sectional study designed to evaluate the effects of military jet fuel on the human immune system. The aim of this study was to determine if changes in the number of white blood cells or constituent components could be detected in the peripheral blood that may indicate abnormalities within the immune system. Particular attention was devoted to lymphocyte subpopulations (T-cells, T-helper cells, T-suppressor cells, NK cells, B-cells), as these were the focus of several animal studies. This study was part of a larger U.S. Air Force research project (The Acute Effects of JP8 Jet Fuel).[7]

Population

Three Air Force bases in the Southeastern United States with significant numbers of personnel performing tank entry work were identified. Volunteers were solicited among tank entry personnel and other low and unexposed base personnel. A recruitment briefing was conducted at each aircraft fuel-systems-repair shop and a letter was sent to all potentially

exposed workers describing the study and asking for participation. Volunteers were also solicited at Commanders Calls, informational press releases and advertisements at local base newspapers Each participant was provided an incentive of fifty dollars. Institutional Review Board approval was obtained from the Air Force and University of Cincinnati and informed consents were signed.

To be included in the study, subjects had to be active duty military personnel with a minimum of nine months on their current base. Tank entry personnel had to have one or more hours of tank entry twice a week for at least nine months (validated against shop records). Personnel in the low exposure group had to have minimal exposure to fuel or solvents in the course of their routine work.

Excluded were those using alcohol within 12 hours prior to entering the study, suffering an injury requiring medical attention within the last six months, having a history of cancer, cerebral vascular accident, diabetes, or seizures on medical profile, pregnant, or using hypertension medication, steroids, antacids or other heartburn medication, diet pills or other stimulants, tranquilizers or muscle relaxants, antidepressants, psychotherapeutic medication or large doses of megavitamins containing antioxidants.

Of the 189 volunteers, 4 did not meet inclusion criteria and 24 were no-shows on the day of sample collection and testing leaving 161 to participate. Sixty of the volunteers were tank entry workers and 50 participated (4 noneligible and 6 no-shows). After venipuncture consents were obtained, blood samples were collected on 151 personnel for Complete Blood Count (CBC) and included in these were 141 who also had blood samples drawn for flow cytometry testing (10 were excluded due to shipping constraints).

Questionnaire

Each participant completed a questionnaire to determine current and past medical history, age, race, gender, months in present job title, body mass index (BMI), tobacco use, alcohol use, and mental exertion. BMI (weight divided by height^2) was calculated from responses to height and weight inquiries. Tobacco use during the preceding six months was dichotomized into smokers and nonsmokers. Alcohol use was determined by multiplying the average number of reported drinks per day times the average number of days per week alcohol was reportedly consumed. Alcohol use was then categorized as none, light (<10 drinks/week), moderate (10-30 drinks/week) and heavy (>30 drinks/week). Respondents were asked to rate the level of mental exertion on the job on a scale of 1 (lowest) to 9 (highest). Responses were grouped into thirds to facilitate analysis and categorized as mild, moderate, and heavy mental exertion.

Exposure Groups

One of the primary investigators for the larger Air Force study, an epidemiologist, convened a group of co-investigators, which by consensus categorized exposure levels by job title. Tank entry personnel were categorized as the high exposure group. Personnel in non-fuels related job titles (e.g. mechanics and information managers) were categorized as the low exposure group. A moderate exposure group was also identified which consisted primarily of fuel distribution workers. This group could not be statistically separated from the high or low exposure groups so individual investigators were given the option of including this group in a

three way analysis or excluding it altogether. This study on immune system effects does not include the moderate exposure group (n=28).

Another investigator for the larger Air Force study collected passive and breath levels of naphthalene, a surrogate for JP8, on all participants. Passive levels were obtained by a breathing zone air-sampling device using an aluminum cartridge containing Tenax to capture volatile organic compounds on participants performing four hours of job-specific tasks. A sampling kit containing a 75 milliliter glass bulb with caps attached to each end was used to collect pre-task and post-task breath samples. Approximately 30 minutes preceding and 30 minutes following a four-hour task, workers removed the caps and forcibly exhaled into the bulb and replaced the caps. Assays on the collected samples were performed by gas chromatography. The purpose was to determine an internal dose that would account for inhalation, dermal absorption and ingestion. The results of these assays were used to validate the categorization of exposure groups by job title.

Lymphocyte Analysis

To control for diurnal variation, blood samples were collected in ten milliliter heparinized tubes during the post exposure phase of data collection, all within a two-hour window in the early afternoon. T-helper cells have been known to vary by 50% or more depending on the time of day of collection.[19] The specimens were packaged and sent overnight express under room temperature to the Travis Air Force Base Clinical Investigation Laboratory in California. Specimens were analyzed on arrival with the length of time from collection to analysis averaging 24 hours (range 22 – 26).

Specimens were analyzed by flow cytometry. Becton Dickinson Immunocytometry Systems TRUCOUNT^{TM} tubes containing a known quantity of beads were used to determine absolute counts of leukocytes. Fifty microliters of heparinized whole blood were added to two tubes, one containing 20 microliters of antibodies to CD3/CD8/CD45/CD4 and the other 20 microliters of CD3/CD16+CD56/CD45/CD19 antibodies. The tubes were capped, gently vortexed for 5 seconds and incubated for 15 minutes in the dark at room temperature. The tubes were uncapped and 450 microliters of FACS Lysing Solution was added to lyse red blood cells. The tubes were recapped, vortexed for 5 seconds and incubated for 15 minutes in the dark. Samples were then run on a Becton Dickinson Fluorescence-Activated Cell Sorter (FACSCalibur^{TM} flow cytometer) using the MultiSET system and the Lyse/No-Wash technique. The flow cytometer was equipped to detect three-color fluorescence, forward scatter, and side scatter to determine the absolute count of lymphocytes and subpopulations (T-cells, T-helper cells, T-suppressor cells, Natural Killer cells, and B cells). The percent of these cells to total lymphocytes was calculated and the absolute number and percent of T-helper cells and T-suppressor cells comprising the T-cells population was also determined.

White Blood Cell Count and Differential Analysis

Three milliliters of blood was collected in a separate tube for a CBC. The CBC was processed by Coulter counter at each base's local clinical laboratory on the day of collection. The white blood cell count and machine generated differential were determined.

Statistical Analysis

The Fisher's Exact test was used to test statistical associations between exposure groups and categorical covariates. The mean, standard deviation, and range were determined for all outcome variables and continuous covariates. To determine significant differences in the means of the high and low exposure groups a normality test was performed on all outcome variables and those with a normal distribution were evaluated with a student t test and those that were non-normal or represented as percentages by a Wilcoxon rank sum test. Pearson Correlation analyses and Analysis of Variance (ANOVA) were applied to identify confounders that were significantly associated with both outcome variables and exposure levels. An Analysis of Covariance (ANOCOVA) using a general linear model procedure with backward elimination was employed to test the differences in outcome variables between exposure levels, while adjusting for other significant covariates. The SAS system was used for all analyses.

Results

Demographics and Life Style Characteristics

Table 1 lists the demographic characteristics by exposure group. There were 1.7 low exposure subjects for each high exposure enrollee. Differences between exposure groups were noted for tobacco use, race, age, gender and BMI. A disproportionate number of smokers were noted in the high exposure group. African Americans and Hispanics were more common in the low exposure group. Only two females were represented in the high exposure group compared to 13 in the low exposure group, a function of few females being employed as tank entry workers. The high exposure group was significantly younger than the low exposure group, although everyone in the study was less than 45 years old. BMI was higher in the low exposure group probably reflecting the older age of the participants. Alcohol consumption, mental exertion and months performing within the current job title did not differ among exposure groups. Therefore, on the variables that the two groups differ, an adjustment was performed in analysis.

Exposure Levels

Both passive Industrial Hygiene (IH) measures and post-task breath analysis of naphthalene differed significantly between exposure groups, which validated the characterization of exposure levels by job title (Table 2). The high exposure group was exposed to mean levels of 583.23 micrograms/m^3 and the low exposure group to 2.47 micrograms/m^3. The post breath levels of 3.80 micrograms/m^3 in the high exposure group were considerably less than the environmental level. The pre-task baseline breath analysis shows no significant difference between exposure groups indicating that high and low exposure personnel had a similar baseline exposure prior to performing their job on the day of testing. Each exposure group had mean levels around 0.75 micrograms/m^3.

Lymphocytes and Subpopulations

Flow cytometry results from 22 blood samples had to be discarded due to quality control issues (Lyse Wash protocol was used instead of Lyse No-Wash) leaving a total of 93 samples for statistical analysis. Table 3 lists results for the lymphocyte analysis. No significant differences were noted between high and low exposure groups.

White Blood Cells and Differential Counts

All 123 samples were adequate for evaluation. White blood cells and differential results are noted in Table 4. Significant elevations of white blood cell counts (p= 0.004), neutrophil counts (p=0.003), and monocyte counts (p=0.02) were noted in the high exposure group versus the low exposure group. After controlling for confounders (smoking and race) and other significant covariates (age, gender, and BMI), significant levels for these same outcome variables persisted; white blood cells (p=0.01), neutrophils (p=0.05) and monocytes (p=0.02).

Discussion

Though the primary Air Force study was related to the acute effects of jet fuel, this study was aimed at the potential health effects of chronic exposure. Tank entry workers in the study were exposed to jet fuel twice a week for at least nine months prior to being studied.

Unlike previous animal studies, no effect on the peripheral blood T-cells was seen on flow cytometry analysis. It is difficult to compare jet fuel levels with naphthalene levels and correlate the inhalation exposures in this study but tank entry personnel and the experimental mice may have had comparable inhalation exposures to JP8. It took inhalation exposures of 250mg/m^3 (one hour for seven days) to decrease T-cell percentages in the animal's peripheral blood.[2] Attendant workers in other studies have been reported to have 15 min STELs of 250mg/m^3 and 8 hour TWAs of 200mg/m^3.[5] The entrant worker wearing a respirator would not be expected to have significant inhalation exposure.[20]

Dermal exposure however, appeared by observation to be substantial as the cotton clothes worn by tank entry workers and attendants were commonly drenched with JP8. Elevated post breath levels in this study suggest dermal absorption. It has been estimated that 100 milliliters absorbed and deposited on the skin of a 200 pound person is equivalent to the exposure level that produced immunotoxic effects in mice.[18] Tank entry workers almost certainly experienced this level of exposure but no change in lymphocyte numbers were noted.

The complete blood count analysis done by Coulter counter showed increased white blood cell numbers, neutrophil counts and monocyte counts. The increase in white blood cells is a function of the increased neutrophils and monocytes. Neutrophils and monocytes are "professional" phagocytic cells.[21] Neutrophils have a half life of about 6-20 hours in the peripheral blood and have the main task of ingesting bacteria although they are capable of binding and ingesting any appropriately opsonized material.[22] The neutrophil is a critical effector cell in humoral and innate immunity and plays vital roles in phagocytosis and bacterial killing.[23] Monocytes in the circulating blood are transformed into macrophages in tissues, such as lung, liver, spleen, lymph nodes and skin. In the lung they are known as alveolar macrophages and in the skin as histiocytes and Langerhans cells. Macrophages can ingest solutes by pinocytosis and larger particles or microbes by phagocytosis.[21] Macrophages and lymphocytes are the most significant cells of the immune system because of their release of lymphokines and cytokines that have wide ranging effects on host defense.

The reason for the elevations of neutrophils and monocytes is not clear. Smoking has been known to be associated with increased white blood cell counts.[24] In this study, elevated WBC counts persisted after adjusting for smoking. It cannot be explained by illness as exclusion

criteria eliminated anyone with a significant medical condition or anyone ill on the day of testing. There have been reports of microbial (bacterial and fungal) colonization of jet fuel.[25] An inhalation exposure to bacteria, endotoxin, fungus or mycotoxin could possibly elevate the neutrophil and monocyte counts. The jet fuel would have to be aerosolized in order for that to happen, however. Exposure to vapors would not be sufficient. In observing tank entry workers involved in job specific tasks, there were no grinding or blowing operations that would produce an aerosol. With intact skin, microbes should not enter the body to produce a systemic reaction that would elevate the white cell count.

It may be that jet fuel vapors create a systemic immune response unrelated to biological agents. An elevated white blood cell count has been observed in inhalation fever, a condition that can occur as the result of inhaling microorganisms but also by inhaling metal fumes, organic grain dust or pyrolysis products of fluoropolymers. The mechanism seems to be related to biochemical messengers mediating a systemic reaction.[26] In dermal exposure, fuel that is absorbed through the skin would almost certainly be ingested by Langerhans cells. These cells would proliferate and other macrophages would be recruited, and if the fuel load were great, the overall response would be increased numbers of monocytes entering the blood from the bone marrow. It is uncertain what the role of the neutrophil would be in a non-microbial foreign substance exposure.

It must be made clear that the increase in white blood cells, neutrophils and monocytes in the high exposure group was only in comparison to the low exposure group. The levels noted were almost always within the normal limits noted in Table 5. In reality, only four of the 123 enrolled subjects had elevated counts of one or more of these three lab tests. All four were in the high exposure group and were scattered among all three bases visited. These abnormalities were noted in individuals who, on the day of the study, denied present illness, significant medical history or medication use. One person had elevations of all three parameters that were also the highest levels seen in each category (WBC 10,200, neutrophils 7,800, and monocytes 1,100). These were not thought to be extreme enough to eliminate the subject from the study.

Limitations

Subjects were not randomly selected. Selection bias could have occurred with the use of volunteers. However it must be noted that almost all tank entry workers available for testing, volunteered, and were accepted into the study.

In cross-sectional studies, such as this one, associations can be drawn but causation cannot be determined. There was, however, significant control of covariates in the attempt to neutralize confounders.

Some of the subjects had to be eliminated from the lymphocyte analysis due to laboratory errors. This should have made little difference, as the percentage decrease in subjects was proportional among high and low exposure groups.

Immune cell counts can give an indication of the intactness of the immune system but does not measure the ability of these cells to function. Future studies should be directed at

mitogen stimulation and proliferation assays that measure function and the ability to produce cytokines that regulate the immune system.

Table 1. Demographic and Life Style Characteristics by Exposure Group

n=123	High Exposure	Low Exposure	p value
	n (%)	**n (%)**	
Subjects	45 (36)	78 (64)	
Tobacco			**0.02**
Smoker	21 (50)	21 (27)	
Nonsmoker	21 (50)	57 (73)	
Alcohol			0.41
None	18 (43)	23 (29)	
Light	5 (12)	13 (17)	
Moderate	18 (43)	41 (53)	
Heavy	1 (2)	1 (1)	
Race (includes Hispanic)			**0.02**
Caucasian	39 (87)	51 (65)	
African American	2 (4)	17 (22)	
Asian/Pacific Islander	1 (2)	1 (1)	
Other	3 (7)	9 (12)	
Hispanic*			1.0
Hispanic	4 (33)	8 (67)	
Gender			**0.05**
Male	43 (96)	65 (83)	
Female	2 (4)	13 (17)	

Mental Exertion			0.61
Mild	16 (37)	34 (44)	
Moderate	21 (49)	36 (47)	
Heavy	6 (14)	7 (9)	
Age	**Years**	**Years**	
Mean	23 .87	27.14	**0.002**
Std dev	4.30	6.17	
Range	18-37	19-44	
Months on job	**Months**	**Months**	
Mean	47.20	50.04	0.75
Std dev	44.11	49.20	
Range	7-172	1-223	
Body Mass Index (BMI)	**BMI**	**BMI**	
Mean	24.61	25.79	**0.04**
Std dev	3.24	2.87	
Range	18-31	19-33	

*Hispanic represents responses to 'also Hispanic' among respondents to questions regarding race.

P values for tobacco, alcohol, race, gender, Hispanic, and mental exertion calculated using Fisher's Exact test. P values for age, month on job, and BMI calculated with student t test.

Significant ($p \leq 0.05$) levels are highlighted in bold.

Table 2. Breathing Zone and Breath Test Naphthalene Levels by Exposure Group

n=123	High Exposure	Low Exposure	p value
Subjects	(n=45)	(n=78)	
Industrial Hygiene			
Mean	583.23	2.47	**<0.0001**
Std dev	268.89	1.73	
Range	123-1000	0.67-8.8	
Pre-Breath			
Mean	0.75	0.71	0.76
Std dev	0.91	0.49	
Range	0.33-6.1	0.33-2.8	
Post-Breath			
Mean	3.80	0.80	**<0.0001**
Std dev	2.17	0.80	
Range	0.9-11	0.33-6.9	

Units – micrograms/cubic meter

Industrial Hygiene - breathing zone passive measures of naphthalene.

Pre-Breath – breath test measures of naphthalene pre-job exposure.

Post -Breath – breath test measures of naphthalene post-job exposure.

P values calculated using student t test.

Significant ($p<0.05$) levels are highlighted in bold.

Table 3. Lymphocyte Subpopulation Counts and Percentages by Exposure Group

n=93	High Exposure		Low Exposure		p value	
	n=36		n=57			
	Counts	Percents	Counts	Percents	Counts	Percents
Total lymphocytes						
Mean	2,041		2,065		0.85	
Std dev	524		624			
Range	1,019-3,245		962-3,658			
T-cells						
Mean	1,520	74.19	1,509	72.88	0.91	0.28
Std dev	423	5.75	490	5.89		
Range	632-2,574	61-84	655-2,866	50-84		
T-sup cells						
Mean	550	27.22	545	26.09	0.92	0.19
Std dev	178	5.95	260	6.69		
Range	179-1,050	15-39	196-1,633	15-46		
T-help cells						
Mean	924	45.30	914	44.72	0.87	0.87
Std dev	283	6.58	284	5.65		
Range	409-1,439	31-60	443-1,556	28-54		
NK cells						
Mean	182	9.08	191	9.33	0.68	0.44
Std dev	96	4.43	95	3.67		
Range	38-480	4-19	32-585	3-22		

B-cells						
Mean	316	15.4	344	16.63	0.39	0.29
Std dev	119	4.2	166	5.45		
Range	122-663	6-28	88-1,105	5-36		

T-sup – T-suppressor cells, T-help – T-helper cells, NK – Natural Killer Cells

T-sup cells and T-help cells are percentages of T-cells. T-cells, NK cells, and B-cells are percentages of Total Lymphocytes.

P values calculated with student t test or Wilcoxon rank sum test.

Counts are cells/mm^3.

Table 4. White Blood Cell Differential Counts and Percentages by Exposure Group

n=123	High Exposure		Low Exposure		p value	
	n=45		N=78			
	Counts	Percents	Counts	Percents	Counts	Percents
White blood cells						
Mean	6,515		5,755		**0.004**	
Std dev	1,402		1,309			
Range	3,100-10,100		3,100-9,000			
Neutrophils						
Mean	3,960	59.65	3,328	57.16	**0.003**	0.33
Std dev	1,267	9.17	1,030	8.90		
Range	1,500-7,800	39-82	1,400-6,400	33-79		
Lymphocytes						
Mean	1,827	28.74	1,799	31.67	0.75	0.11
Std dev	482	7.06	587	8.13		
Range	1,000-3,200	11-41	100-3,700	13-56		
Monocytes						
Mean	518	8.06	440	7.76	**0.02**	0.29
Std dev	193	2.55	155	2.28		
Range	200-1,100	2-14	100-900	2-13		
Eosinophils						
Mean	196	3.06	113	2.93	0.18	0.58
Std dev	165	2.07	125	2.15		
Range	0-500	0.5-11	0-600	0.5-11		

Basophils						
Mean	16	0.49	12	0.48	0.53	0.84
Std dev	37	0.39	32	0.41		
Range	0-100	0-1.7	0-100	0-1.9		

P values calculated with student t test or Wilcoxon rank sum test.

Significant ($p \leq 0.05$) levels are highlighted in bold and represent levels of significance before adjusting for covariates.

Counts are cells/mm^3.

Table 5. Normal Values for White Blood Cell Indices*

	Number of cells/mm^3	
White Blood Cells	3,000-9,400	**Percent of WBC**
Neutrophils	1,000-6,400	40.2-75.4
Monocytes	200-800	4.2-12.6
Eosinophils	0-400	0-6.1
Basophils	0-100	0-1.3
Lymphocytes	800-2,800	14.9-45.8
		Percent of Lymphocytes
Natural Killer cells	90-590	5-27
B-cells	90-660	6-25
T-cells	690-2540	55-84
		Percent of T-cells
T-suppressor	190-1140	13-41
T-helper	410-1590	31-60

*Normal values are those cited by clinical laboratories performing analyses for this study.

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Protein Adducts as Biomarkers of Exposure to Jet Fuel

Introduction:

Use of macromolecular adducts, especially those formed by reactions of electrophilic metabolites with the blood proteins, hemoglobin and serum albumin is widely accepted as an important tool in human biomonitoring. [1-4]. Since protein adducts accumulate over the life of the protein (weeks to months), they provide information about exposure and the systemic availability of reactive metabolites over relatively long periods. These are distinct advantages over short-term biomarkers such as urinary metabolites that reflect exposures during the last few hours and provide no information about systemic availability of reactive metabolites.

Naphthalene is an aromatic hydrocarbon which constitutes about 0.26 % of the hydrocarbon content of JP8 [5]. Naphthalene exposure has been related to a range of health outcomes such as hemolytic anemia and cataracts [6,7]. Since naphthalene can easily be measured in air we hypothesized that this compound would be a useful surrogate for exposure to JP8 [8]. In this study we are evaluating the possibility that protein adducts of naphthalene can be used as long term biomarkers of JP8 exposure. Other parts of this project are evaluating the utility of short-term biomarkers of naphthalene exposure, i.e., naphthalene in breath, and naphthalene and its metabolites in urine.

Naphthalene is metabolized in the liver by cytochrome P450 1A1 and 2A1 to several reactive metabolites, including naphthalene oxide, 1,2- and 1,4-naphthoquinones (NQs), all of which are capable of forming protein adducts. Based upon previous work in our laboratory with benzene [9], which undergoes similar metabolism, we developed an assay to measure cysteinyl adducts of 1,2- and 1,4-NQ with hemoglobin and albumin (1,2-NQ-Ab and 1,4-NQ-Ab). This assay was modified and applied, under protocol D, to 49 control subjects (exposed = 2) and 55 JP8 exposed (exposed = 1) subjects in order to evaluate the possibility using hemoglobin and albumin adduts of 1,2- and 1,4-NQ as long-term biomarkers of exposure to JP8.

Methods:

Albumin and hemoglobin adducts of 1,2- and 1,4-NQs: Blood samples were drawn from workers as mentioned in General Methods, separated to serum and red cells and were shipped to UNC on dry ice. Samples were stored at -80 °C until further use. Albumin and hemoglobin were isolated from serum and red cells, respectively, and were assayed for NQ adducts using a modification of the method of Waidyanatha *et al.* ([9] . Briefly, to 5 mg of albumin or hemoglobin in a 4-ml vial, 5 µg of a mixture containing isotopically-labeled bound internal standards ($[^2H_5]$1,4-NQ-Ab, $[^2H_5]$1,2-NQ-Ab) in 10 mM ascorbic acid/10 mM desferoxamine was added. The samples were brought to dryness and reacted with 750 µl of TFAA and 20 µl of methanesulfonic acid at 100°C for 40 min. The unreacted anhydride was removed under a gentle stream of N_2, samples were reconstituted in 1 ml of hexane and were washed once with 1 ml of 0.1 M Tris buffer (pH 7.2) followed by twice with 1 ml deionized water. After concentrating the samples to 200 µl, a 2 µl-aliquot was analyzed by GC-MS in negative ion chemical ionization mode.

Analyses of Data.

All statistical analyses were performed using Microsoft Excel (Redmond, WV) with a significance level of 0.05 (two-tailed). In light of the highly skewed distributions, all analyses were carried out using (natural) logarithmic transformation of the data. ANOVA was conducted to test the difference between exposed (exposed = 1) and control (exposed = 2) subjects. Least-squares regression was used to investigate the relationships between 1,2-NQ-Ab, 1,4-NQ-Ab and external naphthalene exposure (pass_N).

Status:

Analysis of albumin for 1,2- and 1,4-NQ adducts (in all subjects from DAV and HUR Air Force bases) is completed under protocol D, on 49 control subjects and 56 JP8 exposed subjects in order to validate the use of protein adducts of 1,2- and 1,4-NPQ as biomarkers of exposure to JP8. Preliminary analysis indicate that there are no detectable levels of these adducts in hemoglobin.

Findings:

As mentioned in the methods section, Albumin from 49 control and 56 exposed subjects were analyzed for 1,2-NQ- and 1,4-NQ-Ab adducts. Preliminary analyses indicate that the mean 1,2-NQ-Ab levels in exposed subjects (117 pmol/g of Ab) were slightly higher than the control subjects (86 pmol/g of Ab) ($p = 0.054$). However, 1,4-NQ-Ab levels in exposed subjects (91 pmol/g) were not different from control subjects (103 pmol/g) ($p = 0.201$). There was a weak, but significant, relationship between naphthalene exposure (pass_N, $\mu g/m^3$) and 1,2-NQ-Ab as shown in Figure 1 ($R^2 = 0.076$, $p = 0.009$) while the relationship between1,4-NQ-Ab and exposure was insignificant (Figure 2) ($R^2 = 0.0072$, $p = 0.424$).

Discussion/Conclusions:

In order to evaluate the possibility of using protein adducts of 1,2- and 1,4-NQ as long-term biomarkers of exposure to JP8, hemoglobin and albumin from 56 JP8-exposed subjects and 49 controls were assayed. These adducts were not detected in hemoglobin. Preliminary analysis indicated that 1,2-NQ-Ab in exposed subjects was slightly higher than in the control subjects ($p = 0.054$). However, 1,4-BQ-Ab in the two groups was not statistically different. The correlation of 1,2-NQ-Ab with external naphthalene exposure was weak, but significant ($R^2 = 0.076$, $p = 0.009$). Since albumin adducts integrate exposure over it's lifetime (albumin half-life in humans is 21 d), and daily exposure is highly variable, we would not expect to see a strong correlation between adduct levels and exposure measured only on one day. Hence, average exposures over several days are necessary to validate the relationship between exposure and adduct levels. These data show the potential of 1,2-NQ-Ab as a long-term biomarker of exposure to naphthalene and hence to JP8.

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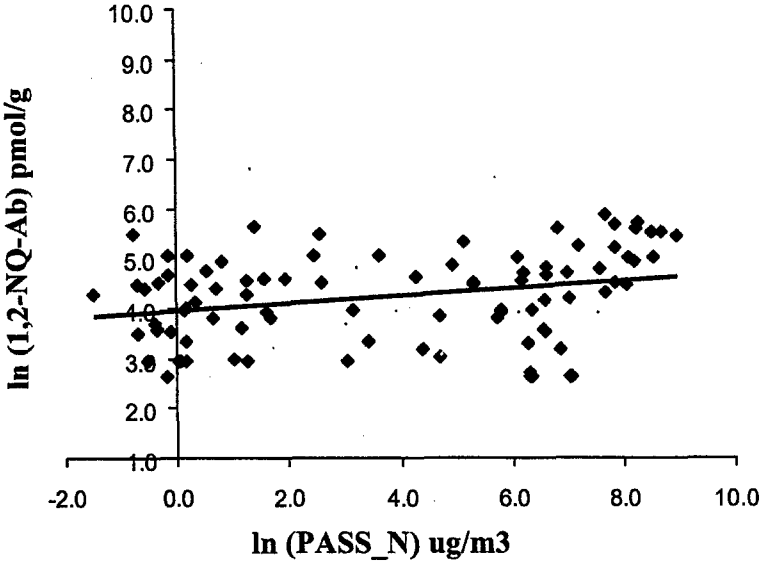

Figure 1. Relationship between log-transformed 1,2-NQ-Ab (pmol/g) and log transformed naphthalene exposure ($\mu g/m^3$). [ln (y) = 0.075 ln (x) + 4.95, R^2 = 0.076, p = 0.009]

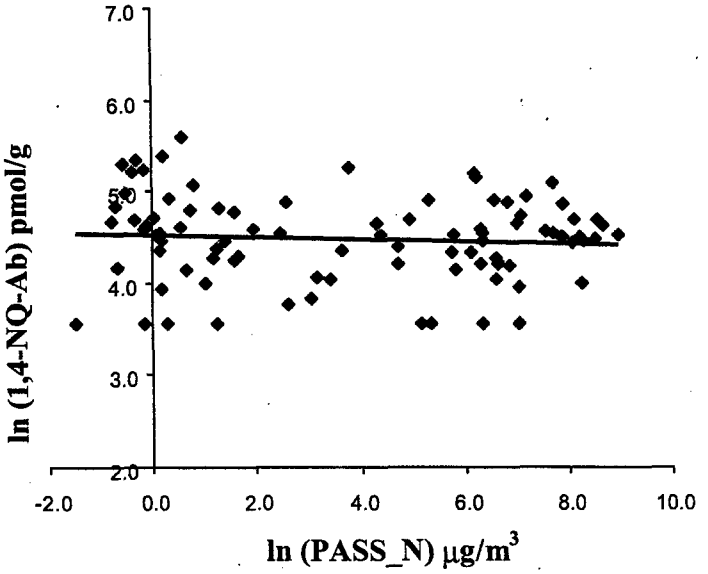

Figure 2. Relationship between log-transformed 1,4-NQ-Ab (pmol/g) and log transformed naphthalene exposure ($\mu g/m^3$). [ln (y) = 0.012 ln (x) + 4.53, R^2 = 0.007, p = 0.423]

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Health Events Comparisons
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Toxicological studies have provided evidence of neurologic, dermatologic, immunologic, cytotoxic, and genotoxic effects from JP8 exposure in animals. Previous research in humans has noted changes in balance and hormone level among those exposed to JP8. Based on these findings, workers exposed to jet fuel may be expected to present for health care in greater numbers than workers of a similar age who do not work with jet fuel. The types of illnesses may range from injuries to infectious disease or cancer. In this study, we reviewed medical record information to determine if differences exist in health care encounter rates when JP8 exposed workers were compared to those who do not routinely encounter jet fuel in the performance of their duties.

Methods:

The study was conducted in two parts. In part one, the medical records of subjects enrolled in the Risk Assessment of Acute Exposure to Jet Fuel protocol were reviewed by a group of epidemiologists. Health visit information was recorded for medical visits occurring within one year of the review date using a disease non-battle injury classification criteria created by the Department of Defense for use in contingency operations. Only initial illness and injury visits were recorded. Follow-up medical appointments, preventive (well) medicine visits and health education or promotion encounters were ignored. The data were analyzed to compare the number of total visits and disease-category-specific encounters among subjects stratified by the revised exposure categories outlined in the General Methods section. Those in the HI category routinely perform duties associated with aircraft fuel systems repair. Those in the MOD category may come into contact with jet fuel as part of their duties. Those in the LOW category do not normally come in contact with jet fuel or other solvents while performing their jobs. Analysis compared the mean number of visits as well as the total number of healthcare visits for three various exposure groups using ANOVA and ANCOVA statistics.

In part two, under a separate protocol, the health outcomes of Active Duty Air Force (ADAF) members whose duties involve working with jet fuel were compared to the health outcomes of those ADAF members with duties that include minimal or no exposure to jet fuel. Using data obtained from the Air Force Personnel Center (AFPC) 5,706 individuals (242 Women; 5464 Men) were identified with potential occupationally related JP8 exposure. From a cohort of 20,224 JP8 occupationally unexposed ADAF members, a random sample of 5,706 subjects were randomly selected using a 1:1 gender-based, frequency matching methodology.

Exposure status for the subjects in the sample was determined by the duty Air Force Specialty Code (AFSC) assigned as of 31 December 1998. The Standard Inpatient Data Records (SIDR) and the Standard Ambulatory Data Records (SADR) databases were queried to identify all inpatient and outpatient visits between January, 1998 and September, 2000 for the study population. The average number of total visits (with and without *well visits*), the average number of *well visits*, and the average number of visits by reason for the visit for the exposed and unexposed groups, stratified by gender, were compared using the Student's t-test at the $\alpha = .05$

level. For initial analysis, the exposure status was dichotomized into exposed and unexposed categories. All ADAF personnel with AFSCs representing Aircraft Fuel Cell Maintenance, Re-fuelers, Fuel Vehicle Maintenance workers, and those working with Petroleum, Oil, and Lubricants were selected for the exposed group. The unexposed group included those ADAF members who worked in Supply, Civil Engineering, Personnel, Administration (Finance), and Services (Cooks). The comparisons were re-analyzed with the exposure variable further separated into five ordinal levels of exposure. The categories were labeled from 0 (No Exposure) to 4 (Most Exposure) with the following categorization: 0 – Personnel/Administration; 1 – Services and Supply; 2 – Civil Engineers; 3 – Re-fuelers, Fuel vehicle maintenance workers, and those who work with Petroleum, Oil and Lubricants; and 4 – Fuel Cell Workers.

In both protocols, those routinely exposed to JP8 on-the-job, had similar health care visit rates to those who do not come into contact with jet fuel. In the enrolled subjects protocol, the small sample size and modest medical record availability rate (Males 77%, Females 81%) yielded rather unstable results. In the amply powered electronically derived health care visit protocol, the data showed no association between JP8 exposure classification and health care visit rates. The tables accompanying this abstract provide gender-stratified comparisons for specific disease categories for both analyses. After accounting for multiple comparisons in the analysis, none of the results from either protocol were statistically significant.

Health Encounters from Medical Record Review
Enrolled Subjects

	Males = 220			Females = 45		
Mean Number of Events	**HI**	**MOD**	**LOW**	**HI**	**MOD**	**LOW**
Skin Diseases	0.3	0.29	0.34	0.4	0	0.36
Gastrointestinal Concerns	0.1	0.05	0.15	0.6	0	0.36
Sports related Injuries	0.13	0.18	0.15	0	0	0.27
Workplace Injuries	0.08	0.08	0.08	0	0.06	0.06
Other Injuries	0.11	0.09	0.08	0.3	0	0.06
Total Injuries	0.32	0.35	0.31	0.4	0	0.39
Respiratory Conditions	0.6	0.32	0.51	0.6	5	1.33
Neurological Conditions	0.1	0.12	0.19	0.2	0	0.24
Musculoskeletal Conditions	0.31	0.29	0.35	0.6	0.5	0.3
Cardiovascular Conditions	0.03	0	0.07	0	0	0
Urogenital Complaints	0.1	0.03	0.09	0.2	0.5	0.8
Total Visits	1.95	1.62	2.09	3.1	2.0	3.85

JP8 Health Effects
Data Derived from Electronic Medical Records Systems

MALES

Number of Visits (Mean)	Fuel Cell Workers	Personnel/Admin	Civil Engineering	Services/Supply	Refuelers/Petro Workers
Total Visits	11.71	10.37	12.09	10.21	10.07
Well Visits	5.37	3.60	4.71	4.05	4.33
Sick Call Visits	6.34	6.77	7.38	6.16	5.74
Infectious/Parasitic Illnesses	0.36	0.40	0.38	0.40	0.35
Neoplasms	0.07	0.07	0.09	0.09	0.06
Endocrine	0.14	0.19	0.18	0.14	0.17
Blood Related Illnesses	0.13	0.09	0.02	0.01	0.01
Mental Illness	0.49	0.43	0.40	0.35	0.36
Neurological Illnesses	0.58	0.71	0.76	0.64	0.54
Circulatory System	0.21	0.29	0.26	0.29	0.23
Respiratory Illnesses	0.81	0.90	0.98	0.73	0.74
Digestive Related Illnesses	0.30	0.30	0.29	0.26	0.28
Genitourinary Related Illnesses	0.11	0.14	0.13	0.12	0.12
Dermatological Related Illnesses	0.35	0.40	0.39	0.37	0.33
Musculoskeletal	1.48	0.16	1.93	1.52	1.27
Congenital	0.01	0.01	0.01	0.01	0.01
Injury Related	0.82	0.85	1.06	0.73	0.82

JP8 Health Effects
Data Derived from Electronic Medical Records Systems

FEMALES

Number of Visits (Mean)	Fuel Cell Workers	Personnel/Admin	Civil Engineering	Services/Supply	Refuelers/Petro Workers
Total Visits	22.08	20.22	NA*	20.33	18.53
Well Visits	9.94	9.94	NA*	8.99	7.78
Sick Call Visits	12.13	12.13	NA*	11.34	9.94
Infectious/Parasitic Illnesses	0.54	0.48	NA*	0.63	0.77
Neoplasms	0.12	0.16	NA*	0.75	0.17
Endocrine	0.50	0.25	NA*	0.14	0.25
Blood Related Illnesses	0.05	0.01	NA*	0.01	0.02
Mental Illness	0.93	1.06	NA*	1.76	0.60
Neurological Illnesses	0.81	1.15	NA*	0.95	0.91
Circulatory System	0.02	0.12	NA*	0.22	0.07
Respiratory Illnesses	1.35	1.31	NA*	0.86	1.39
Digestive Related Illnesses	0.35	0.39	NA*	0.37	0.48
Genitourinary Related Illnesses	0.93	1.23	NA*	0.87	0.88
Dermatological Related Illnesses	0.55	0.58	NA*	0.67	0.56
Musculoskeletal	3.28	3.28	NA*	1.80	2.34
Congenital	0.00	0.02	NA*	0.07	0.05
Injury Related	1.46	0.78	NA*	0.76	1.10
Pregnancy Related	0.26	0.31	NA*	0.26	0.17
Perinatal Related	0.01	0.00	NA*	0.08	0.00

NA* = No Female Civil Engineering Females were Selected

Discussion/Conclusions:

These findings indicate that active duty Air Force workers exposed to JP8 are no more likely to seek medical attention for a variety of health concerns than workers who are not exposed to JP8 on the job. In both methods of collecting health care information and across multiple comparison groups, workers exposed to JP8 in most instances had basically the same condition-specific health visits as controls. While the data used to conduct this analysis may be subject to condition-specific coding errors in some cases; particularly the data derived from ADS electronic records, any misclassification is expectedly non-differential and therefore unlikely to change the direction of the noted effects. In other words, any inherent non-differential misclassification bias in these data would not be expected to change the relationship from protective to causal with respect to JP8 exposure and health event frequency.

Several potential biases may have affected these results. There may be underlying differences in health care seeking behavior between JP8 exposed and unexposed groups. If JP8 exposed workers are less likely to seek health care either in general or due to job constraints or other mitigating issues, our analysis could show no effect in spite of true underlying difference is disease incidence. However, a review the ADS data for total health care visits (including well visits) shows JP8 exposed workers had a similar rate of health encounters as the comparison cohorts. While condition-specific differences in health care seeking behavior can not be ruled out as a reason for the findings noted in this analysis, findings relative to total health care visits would indicate that differences in health care seeking behavior do not play a pivotal role in these findings. The limited time frame from which health encounter information was derived in both protocols is also a limiting factor. Because the data cover only 1 year or 18 month period, temporal trends in health care can not be determined. Additionally, health encounter information from either medical record review or electronic records is, by nature, severity based. Fuel cell workers may be subject to more minor ailments that other workers but not to more severe conditions, which would result in soliciting professional medical help. Conditioning or "hardening" to exposure-associated conditions and potential differential applications of self-aid/buddy care may also play a role in these findings. Further, the analysis does not specifically address chronic health concerns such as heart disease, degenerative joint disease or cancer. Military members in general and JP8 workers in particular represent a young worker population. Further study is needed to investigate the impact of JP8 exposure on chronic disease.

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Summary for Website - Health Events Comparisons
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Toxicological studies have provided evidence of neurologic, dermatologic, immunologic, cytotoxic, and genotoxic effects from JP8 exposure in animals. Previous research in humans has noted changes in balance and hormone level among those exposed to JP8. Based on these findings, workers exposed to jet fuel may be expected to present for health care in greater numbers than workers of a similar age who do not work with jet fuel. The types of illnesses may range from injuries to infectious disease or cancer. In this study, we reviewed medical record information to determine if differences exist in health care encounter rates when JP8 exposed workers were compared to those who do not routinely encounter jet fuel in the performance of their duties.

Methods:

The study was conducted in two parts. In part one, the medical records of subjects enrolled in the Risk Assessment of Acute Exposure to Jet Fuel protocol were reviewed by a group of epidemiologists. Health visit information was recorded for medical visits occurring within one year of the review date using a disease non-battle injury classification criteria created by the Department of Defense for use in contingency operations. Only initial illness and injury visits were recorded. Follow-up medical appointments, preventive (well) medicine visits and health education or promotion encounters were ignored. The data were analyzed to compare the number of total visits and disease-category-specific encounters among subjects stratified by the revised exposure categories outlined in the General Methods section. Those in the HI category routinely perform duties associated with aircraft fuel systems repair. Those in the MOD category may come into contact with jet fuel as part of their duties. Those in the LOW category do not normally come in contact with jet fuel or other solvents while performing their jobs. Analysis compared the mean number of visits as well as the total number of healthcare visits for three various exposure groups using ANOVA and ANCOVA statistics.

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In both protocols, those routinely exposed to JP8 on-the-job, had similar health care visit rates to those who did not come into contact with jet fuel. In the enrolled subjects protocol, the small sample size and modest medical record availability rate (Males 77%, Females 81%) yielded rather unstable results. In the amply-powered electronically derived health care visit protocol, the data showed no association between JP8 exposure classification and health care visit rates. After accounting for multiple comparisons in the analysis, none of the results from either protocol were statistically significant.

Discussion/Conclusions:

These findings indicate that active duty Air Force workers exposed to JP8 are no more likely to seek medical attention for a variety of health concerns than workers who are not exposed to JP8 on the job. In both methods of collecting health care information and across multiple comparison groups, workers exposed to JP8 in most instances had basically the same condition-specific health visits as controls. While the data used to conduct this analysis may be subject to condition-specific coding errors in some cases (particularly the data derived from ADS electronic records), any misclassification is expectedly non-differential and therefore unlikely to change the direction of the noted effects. In other words, any inherent non-differential misclassification bias in these data would not be expected to change the relationship from protective to causal with respect to JP8 exposure and health event frequency.

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Self Reported Health Status
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Military members who routinely work with JP8 have expressed concerns regarding the impact of fuel exposure on their health; both acute health problems and long-term health effects. Toxicological studies of JP8 have demonstrated exposure to high levels of JP8 via either dermal contact or inhalation may result in adverse neurologic, immunologic, dermatological, cytotoxic, and genotoxic effects in animals. If worker health is impacted by jet fuel, those working with JP8 could be expected to report more illness symptoms that those who do not work with jet fuel. As part of the Risk Assessment of Acute Exposure to Jet Fuel protocol, we assessed the relative frequency workers reported health concerns using a questionnaire.

Methods:

Subjects enrolled in the Risk Assessment of Acute Exposure to Jet Fuel protocol were asked to complete a questionnaire as part of the study. The questionnaire was applied electronically as part of a larger neurocognitive battery. Subjects were asked to answer 77 questions designed to provide information on health symptoms, personal habits, off-duty exposures, and types of personal protective equipment used at work. The questionnaire data were analyzed to compare the frequency of self-reported health symptoms among subjects stratified by the revised exposure categories outlined in the General Methods section. Those in the HI category routinely perform duties associated with aircraft fuel systems repair. Those in the MOD category may come into contact with jet fuel as part of their duties. Those in the LOW category do not normally come in contact with jet fuel or other solvents while performing their jobs. Analysis compared the rate of responses to specific health related questions among the three exposure groups. Questionnaire responses were regrouped into bivariate categories (i.e. no headaches vs 1 or more headaches in the 6 month period preceding questionnaire application. HI and MOD categories were compared to the LOW category. Risk estimates with 95% confidence intervals and Chi-Square statistics were calculated.

Findings:

Of the 339 subjects enrolled in the study, self-reported health status data were available from 328 individuals. Among males enrolled in the study, those in the HI exposure category generally reported more symptoms that those in the LO category. Specific symptom risk comparisons are provided in the table accompanying this report. Those in the MOD category also tended to report more health related symptoms than those in the LOW group and in several cases more than those in the HI group. In particular, workers exposed to JP8, either routinely or occasionally, were more likely to believe past and current work impacted their health. Those in the HI or MOD categories reported a greater likelihood of having symptoms such as headaches, dizziness, difficulty breathing, chest tightness, heart palpitations, balance problems, walking difficulties, excess sweating, general weakness, trouble concentrating, forgetfulness, and trouble gripping things. They also reported experiencing more blisters on their hands or forearms during the six months preceding questionnaire application. The findings remained significant after accounting statistically for multiple testing using Bonferroni methodology. Female study

participants reported similar findings. These findings were less stable statistically; due to the much small number of females enrolled in the study.

Self Reported Symptoms
% Reporting Symptoms at Least Once in Last 6 Months

	HI	LOW	OR	95% CI
Head Ache	76.1	50.3	3.14	(1.85 - 5.32)*
Dizziness	52.2	17.2	5.23	(3.02 - 9.13)*
Blurry Vision	31.9	19.1	1.98	(1.14 - 3.46)
Itchy Skin	82.8	18.5	21.2	(11.3 - 39.7)*
Difficulty Breathing	29.9	15.3	2.37	(1.31 - 4.26)
Chest Tightness	39.7	17.2	3.16	(1.81 - 5.52)*
Heart Palpitations	19.7	6.4	3.60	(1.64 - 7.89)*
Ringing Ears	54.7	38.2	1.95	(1.20 - 3.17)
Imbalance	45.3	14.0	5.82	(2.85 - 9.07)*
Tremors	44.4	26.8	2.19	(1.32 - 3.64)
Excess Sweating	23.9	9.7	2.94	(1.49 - 5.80)
Teary Eyes	26.7	14.0	2.24	(1.22 - 4.12)
Numbness	31.6	18.5	2.04	(1.16 - 3.58)
Walking Difficulties	16.4	2.5	7.49	(2.48 - 22.69)*
General Weakness	24.8	7.0	4.37	(2.08 - 9.19)*
Chronic Pain	16.4	10.8	1.61	(0.79 - 3.26)
Pain Medication	15.4	11.5	1.40	(0.70 - 2.83)
Trouble Concentrating	33.3	16.6	2.52	(1.43 - 4.45)
Forgetfulness	37.1	17.8	2.71	(1.56 - 4.73)
Balance Problems	21.6	5.1	5.11	(2.21 - 11.83)*
Gripping Things	11.2	6.4	1.85	(0.78 - 4.39)
Life's Work Impacting Health	44.7	16.5	4.11	(2.36 - 7.15)*
Current Job Impacting Health	58.1	13.2	9.15	(5.12 - 16.33)*
Weeping Skin	12.0	7.0	1.80	(0.78 - 4.13)
Scaly Skin	6.0	3.8	1.62	(0.53 - 4.94)
Chemical Allergy	1.7	0.6	2.71	(0.24 - 30.28)
Blisters on Hands/Arm	56.4	13.4	8.38	(4.66 - 15.10)*

* = Statistically significant after accounting for multiple testing

JP8 Acute Exposure Study
Self Reported Symptoms
% Reporting Symptoms at Least Once in Last 6 Months

	MOD	LOW	OR	95% CI
Head Ache	72.7	50.3	2.63	(1.26 - 5.49)
Dizziness	47.7	17.2	4.40	(2.14 - 9.09)*
Blurry Vision	38.6	19.1	2.67	(1.29 - 5.49)
Itchy Skin	61.4	18.5	6.99	(3.38 - 14.49)*
Difficulty Breathing	37.2	15.3	3.28	(1.54 - 6.99)*
Chest Tightness	52.3	17.2	5.26	(2.56 - 10.86)*
Heart Palpitations	32.6	6.4	7.09	(2.87 - 17.54)
Imbalance	43.2	14.0	4.67	(1.35 - 9.80)*
Tremors	50.0	26.8	2.74	(1.38 - 5.46)
Excessive Sweating	31.8	9.7	4.37	(1.90 - 10.0)*
Teary Eyes	22.7	14.0	1.80	(4.16 - 0.78)
Numbness	52.3	18.5	4.80	(2.37 - 9.76)*
Walking Difficulties	16.4	2.5	7.49	(2.48 - 22.69)*
General Weakness	24.8	7.0	4.37	(2.08 - 9.19)*
Chronic Pain	16.4	10.8	1.61	(0.79 - 3.26)
Pain Medication	25.6	12.0	2.53	(1.10 - 5.79)
Trouble Concentrating	39.5	16.6	3.39	(1.78 - 7.09)*
Forgetfulness	47.7	17.4	4.35	(2.13 - 8.88)*
Balance Problems	20.9	4.8	5.26	(1.89 - 14.62)*
Gripping Things	18.6	6.0	3.59	(1.32 - 9.75)*
Life's Work Impacting Health	39.0	16.6	3.25	(1.53 - 6.88)*
Current Job Impacting Health	54.8	15.2	7.98	(3.75 - 16.98)*
Weeping Skin	00.0	1.2	N/A	----------
Scaly Skin	4.5	3.0	1.62	(0.28 - 8.23)
Chemical Allergy	2.3	0.6	3.86	(0.24 - 62.98)*
Blisters on Hands/Arm	56.4	13.4	4.54	(2.12 - 9.75)*

*** = Statistically significant after accounting for multiple testing**

Discussion/Conclusions:

The symptoms reported at highest frequency among those with routine or occasional exposure to JP8 (blisters, itchy skin, dizziness, balance and walking difficulties) are consistent with expected symptoms based on toxicological studies and previously conducted human studies. While self-reported, symptom-based questionnaires applied among non-blinded study subjects are subject to recall and other biases, the fact remains that symptoms reported with greatest frequency and with the strongest statistical significance are among those we would expect to occur among people exposed to jet fuel. Subjects with only occasional exposure to JP8 tended to report more symptoms than those who routinely work with jet fuel for some categories indicating that other occupational exposures may play a part in the self-reported worker health complaints. More importantly, based on the data, jet fuel exposed participants in this study strongly believe their job is impacting their health. This finding supports the need for improved risk communication regarding jet fuel, better adherence to work practice guidelines, and further research into enhanced personal protection equipment

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Summary for Website - Self Reported Health Status
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Military members who routinely work with JP8 have expressed concerns regarding the impact of fuel exposure on their health; both acute health problems and long-term health effects. Toxicological studies of JP8 have demonstrated exposure to high levels of JP8 via either dermal contact or inhalation may result in adverse neurologic, immunologic, dermatological, cytotoxic, and genotoxic effects in animals. If worker health is impacted by jet fuel, those working with JP8 could be expected to report more illness symptoms that those who do not work with jet fuel. As part of the Risk Assessment of Acute Exposure to Jet Fuel protocol, we assessed the relative frequency workers reported health concerns using a questionnaire.

Methods:

Subjects enrolled in the Risk Assessment of Acute Exposure to Jet Fuel protocol were asked to complete a questionnaire as part of the study. The questionnaire was applied electronically as part of a larger neurocognitive battery. Subjects were asked to answer 77 questions designed to provide information on health symptoms, personal habits, off-duty exposures, and types of personal protective equipment used at work. The questionnaire data were analyzed to compare the frequency of self-reported health symptoms among subjects stratified by the revised exposure categories outlined in the General Methods section. Those in the HI category routinely perform duties associated with aircraft fuel systems repair. Those in the MOD category may come into contact with jet fuel as part of their duties. Those in the LOW category do not normally come in contact with jet fuel or other solvents while performing their jobs. Analysis compared the rate of responses to specific health related questions among the three exposure groups. Questionnaire responses were regrouped into bivariate categories (i.e. no headaches vs 1 or more headaches in the 6 month period preceding questionnaire application. HI and MOD categories were compared to the LOW category. Risk estimates with 95% confidence intervals and Chi-Square statistics were calculated.

Findings:

Of the 339 subjects enrolled in the study, self-reported health status data were available from 328 individuals. Among males enrolled in the study, those in the HI exposure category generally reported more symptoms that those in the LO category. Specific symptom risk comparisons are provided in the table accompanying this report. Those in the MOD category also tended to report more health related symptoms than those in the LOW group and in several cases more than those in the HI group. In particular, workers exposed to JP8, either routinely or occasionally, were more likely to believe past and current work impacted their health. Those in the HI or MOD categories reported a greater likelihood of having symptoms such as headaches, dizziness, difficulty breathing, chest tightness, heart palpitations, balance problems, walking difficulties, excess sweating, general weakness, trouble concentrating, forgetfulness, and trouble gripping things. They also reported experiencing more blisters on their hands or forearms during the six months preceding questionnaire application. The findings remained significant after accounting statistically for multiple testing using Bonferroni methodology. Female study participants reported similar findings. These findings were less stable statistically; due to the much small number of females enrolled in the study.

Discussion/Conclusions:

The symptoms reported at highest frequency among those with routine or occasional exposure to JP8 (blisters, itchy skin, dizziness, balance and walking difficulties) are consistent with expected symptoms based on toxicological studies and previously conducted human studies. While self-reported, symptom-based questionnaires applied among non-blinded study subjects are subject to recall and other biases, the fact remains that symptoms reported with greatest frequency and with the strongest statistical significance are among those we would expect to occur among people exposed to jet fuel. Subjects with only occasional exposure to JP8 tended to reported more symptoms than those who routinely work with jet fuel for some categories indicating that other occupational exposures may play a part in the self-reported worker health complaints. More importantly, based on the data, jet fuel exposed participants in this study strongly believe their job is impacting their health. This finding supports the need for improved risk communication regarding jet fuel, better adherence to work practice guidelines, and further research into enhanced personal protection equipment

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JP8 Health Effects

Data Derived from Ambulatory Data System Records

MALES

Number of Visits (Mean)	Fuel Cell Workers	Personnel/Admin	Civil Engineering	Services/Supply	Refuelers/Petro Workers
Total Visits	11.71	10.37	12.09	10.21	10.07
Well Visits	5.37	3.60	4.71	4.05	4.33
Sick Call Visits	6.34	6.77	7.38	6.16	5.74
Infectious/Parasitic Illnesses	0.36	0.40	0.38	0.40	0.35
Neoplasms	0.07	0.07	0.09	0.09	0.06
Endocrine	0.14	0.19	0.18	0.14	0.17
Blood Related Illnesses	0.13	0.09	0.02	0.01	0.01
Mental Illness	0.49	0.43	0.40	0.35	0.36
Neurological Illnesses	0.58	0.71	0.76	0.64	0.54
Circulatory System	0.21	0.29	0.26	0.29	0.23
Respiratory Illnesses	0.81	0.90	0.98	0.73	0.74
Digestive Related Illnesses	0.30	0.30	0.29	0.26	0.28
Genitourinary Related Illnesses	0.11	0.14	0.13	0.12	0.12
Dermatological Related Illnesses	0.35	0.40	0.39	0.37	0.33
Musculoskeletal	1.48	0.16	1.93	1.52	1.27
Congenital	0.01	0.01	0.01	0.01	0.01
Injury Related	0.82	0.85	1.06	0.73	0.82

JP8 Health Effects
Data Derived from Ambulatory Data System Records

FEMALES

Number of Visits (Mean)	Fuel Cell Workers	Personnel/Admin	Civil Engineering	Services/Supply	Refuelers/Petro Workers
Total Visits	22.08	20.22	NA*	20.33	18.53
Well Visits	9.94	9.94	NA*	8.99	7.78
Sick Call Visits	12.13	12.13	NA*	11.34	9.94
Infectious/Parasitic Illnesses	0.54	0.48	NA*	0.63	0.77
Neoplasms	0.12	0.16	NA*	0.75	0.17
Endocrine	0.50	0.25	NA*	0.14	0.25
Blood Related Illnesses	0.05	0.01	NA*	0.01	0.02
Mental Illness	0.93	1.06	NA*	1.76	0.60
Neurological Illnesses	0.81	1.15	NA*	0.95	0.91
Circulatory System	0.02	0.12	NA*	0.22	0.07
Respiratory Illnesses	1.35	1.31	NA*	0.86	1.39
Digestive Related Illnesses	0.35	0.39	NA*	0.37	0.48
Genitourinary Related Illnesses	0.93	1.23	NA*	0.87	0.88
Dermatological Related Illnesses	0.55	0.58	NA*	0.67	0.56
Musculoskeletal	3.28	3.28	NA*	1.80	2.34
Congenital	0.00	0.02	NA*	0.07	0.05
Injury Related	1.46	0.78	NA*	0.76	1.10
Pregnancy Related	0.26	0.31	NA*	0.26	0.17
Perinatal Related	0.01	0.00	NA*	0.08	0.00

NA* = No Female Civil Engineering Females were Selected

A Model For Predicting Health Risk to Exposure to JP8 Jet Fuel

Introduction:

We developed a PBPK model to predict JP8 jet fuel effects on U.S. Air Force fuel cell workers. The model was based on a PBPK model for naphthalene inhalation in mice and rats (U.S.D.H.H.S. 2000). We also implemented a PBPK model of JP8 jet fuel components (nonane) by Robinson (2000), which was based on a PBPK model of inhalation of styrene (Ramsey and Andersen, 1984). A third model that we also examined was another PBPK model of naphthalene inhalation in mice and rats (Sweeney, et al. 1996, Quick and Shuler 1999). All of these models were designed for inhalation exposure only. We added a second pathway for dermal exposure and a skin compartment to both the DHHS and Robinson models (Figure1).

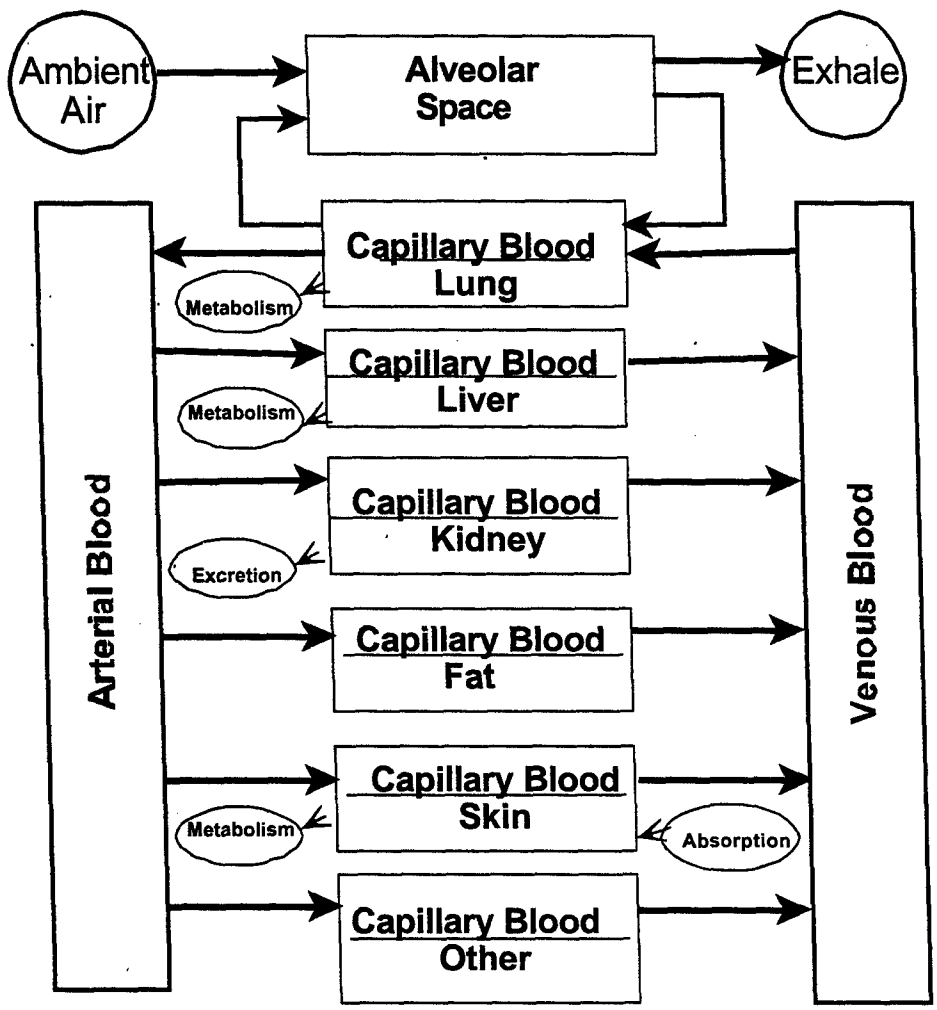

Figure 1 Flow diagram of a PBPK model for naphthalene inhalation and skin absorption (adapted from U.S. Department of Health and Human Services 2000).

The model consists a calculation of the naphthalene concentration in each compartment for each individual in the population. The model, which is diffusion limited, contains compartments including arterial and venous blood, lung, liver, kidney, fat, skin, and other organs (Figure 1). The other organ compartment represents both slowly and rapidly perfused tissue (e.g., muscle, bone, heart, brain). Inhalation of naphthalene form ambient air concentrations takes place through the alveolar space into the lung. Modeled uptake is dependent upon the ventilation rate, permeability of the tissue, and blood flow through the lung. Metabolism of naphthalene was assumed to take place primarily in the liver, but also in the lungs and skin. One metabolic pathway was used in both the lungs and skin, whereas in the liver, two pathways were used – one represented by Michaelis-Menten kinetics and the other by Hill kinetics. Dermal absorption takes place through naphthalene contact with the skin. Population responses were estimated by determining the response of many individuals in a population (defined by Air Force base and sex.). The model is stochastic in that it contains random variables. The random variables in the body burden (dose calculation) are the dermal exposure concentrations and ambient air concentrations. These random variables provide the capability to conduct stochastic simulations.

Methods:

The model consists of a set of ordinary differential equations programmed in the Matlab® programming language (The Mathworks, Inc., Natick, MA). The differential equations were solved using second-order and third-order Runge-Kutta methods with default tolerances.

The equations represent the dynamics of naphthalene as shown in Figure 1. Naphthalene is inhaled from ambient air via the alveolar space (Equation 1) into the lung capillary blood (Equation 3). From the lung capillary blood, it goes either to arterial blood (Equation 2) or to the lung tissue (Equation 4) where it is metabolized. From the arterial blood, it is distributed to the liver (Equations 6 and 7) and skin (Equations 8 and 9) where it also is metabolized, or to other tissues (Equations 10 and 11). Except for the lung capillary space, the effluent from all of the tissue capillary spaces goes to the venous blood compartment (Equation 5). Naphthalene is transported via the venous blood to the lung capillary space (Equation 3).

Symbols used to describe model equations are defined in Table 1. Parameters used in the model simulations are listed in Table 2.

Table 1. Abbreviations and symbols used in describing a PBPK model for naphthalene (adapted from U.S. Department of Health and Human Services 2000).

V	Volume of tissue or blood (mL)
Concentrations:	
AMT_{air}	Amount in inhaled air (mg)
AMT_{alv}	Amount in alveolar air (mg)
AMT_{art}	Amount in arterial blood (mg)
AMT_{ven}	Amount in venous blood (mg)
$AMT_{tissuecap}$	Amount in tissue capillary blood (mg)
AMT_{tissue}	Amount in tissue (mg)
Flows:	
$Qvent$	Alveolar ventilation rate (mL/min)
$Qtotal$	Total blood flow (mL/min)
$Qtissue$	Blood flow to the tissue (mL/min)
Partition coefficients and permeability constant:	
$Perm$	Capillary permeability constant
P_{tissue}	Tissue:blood partition coefficient
P_{air}	Blood:air partition coefficient
Metabolism rates:	
V_{max}	Maximum enzymatic reaction rate (mg/hr)
K_m	Michaelis constant for enzymatic reaction (mg/liter blood)
n	Hill constant

Differential Equations. All equations are from U.S.D.H.H.S (2000) except the skin compartment, which was adapted from the liver compartment with added terms from Krishnan and Andersen (2001).

Alveolar space:

Equation 1

$$\frac{dAMT_{alv}}{dt} = Dose \bullet Q_{vent} + \frac{AMT_{lungcap}}{V_{lungcap}} \bullet \frac{Q_{vent}}{P_{air}} \bullet Perm - \ldots$$
$$\frac{AMT_{alv}}{V_{alv}} \bullet Q_{vent} \bullet Perm - \frac{AMT_{alv}}{V_{alv}} \bullet Q_{vent}$$

Arterial blood:

Equation 2

$$\frac{dAMT_{art}}{dt} = \frac{AMT_{lungcap}}{V_{lungcap}} \bullet Q_{total} - \frac{AMT_{art}}{V_{art}} \bullet Q_{total}$$

Lung:

Equation 3

$$\frac{dAMT_{lungap}}{dt} = \frac{AMT_{ven}}{V_{ven}} \bullet Q_{total} \bullet Perm + \frac{AMT_{alv}}{V_{alv}} \bullet Q_{vent} \bullet Perm + \ldots$$
$$\frac{AMT_{lung}}{V_{lung}} \bullet \frac{Q_{total}}{P_{lung}} \bullet Perm - \frac{AMT_{lungcap}}{V_{lungcap}} \bullet Q_{total} - \ldots$$
$$\frac{AMT_{lungcap}}{V_{lungcap}} \bullet Q_{total} \bullet Perm - \frac{AMT_{lungcap}}{V_{lungcap}} \bullet \frac{Q_{vent}}{P_{air}} \bullet Perm$$

Equation 4

$$\frac{dAMT_{lung}}{dt} = \frac{AMT_{lungcap}}{V_{lungcap}} \bullet Q_{total} \bullet Perm - \frac{AMT_{lung}}{V_{lung}} \bullet \frac{Q_{total}}{P_{lung}} \bullet Perm - \ldots$$
$$\frac{V_{\max lung} \bullet V_{lung} \bullet AMT_{lung}}{K_{mlung} \bullet V_{lung} + AMT_{lung}}$$

Venous blood:

Equation 5

$$\frac{dAMT_{ven}}{dt} = \sum \frac{AMT_{tissuecap}}{V_{tissuecap}} \bullet Q_{tissue} - \frac{AMT_{ven}}{V_{ven}} \bullet Q_{total}$$

Liver:

Equation 6

$$\frac{dAMTlivercap}{dt} = \frac{AMTart}{Vart} \bullet Qliver + \frac{AMTliver}{Vliver} \bullet \frac{Qliver}{Pliver} \bullet Perm - \ldots$$
$$\frac{AMTlivercap}{Vlivercap} \bullet Qliver - \frac{AMTlivercap}{Vlivercap} \bullet Qliver \bullet Perm$$

Equation 7

$$\frac{dAMT_{liver}}{dt} = \frac{AMT_{livercap}}{V_{livercap}} \bullet Q_{liver} \bullet Perm - \frac{AMT_{liver}}{V_{liver}} \bullet \frac{Q_{liver}}{P_{liver}} \bullet Perm - \ldots$$
$$\frac{V_{\max liver} \bullet V_{liver} \bullet AMT_{liver}}{K_{mliver1} \bullet V_{liver} + AMT_{liver}} - \frac{V_{\max liver2} \bullet V_{liver} \bullet AMT_{liver}^{n}}{(K_{mliver2} \bullet V_{liver})^{n} + AMT_{liver}^{n}}$$

Skin:

Equation 8

$$\frac{dAMT_{skincap}}{dt} = \frac{AMT_{art}}{V_{art}} \bullet Q_{skin} + \frac{AMT_{skin}}{V_{skin}} \bullet \frac{Q_{skin}}{P_{skin}} \bullet Perm - \ldots$$
$$\frac{AMT_{skincap}}{V_{skincap}} \bullet Q_{skin} - \frac{AMT_{skincap}}{V_{skincap}} \bullet Q_{skin} \bullet Perm$$

Equation 9

$$\frac{dAMT_{skin}}{dt} = K_p \bullet S \bullet (Dose - AMT_{skin}) + \frac{AMT_{skincap}}{V_{skincap}} \bullet Q_{skin} \bullet Perm - \ldots$$
$$\frac{AMT_{skin}}{Vskin} \bullet \frac{Q_{skin}}{P_{skin}} \bullet Perm - \frac{V_{\max skin} \bullet V_{skin} \bullet AMT_{skin}}{K_{mskin} \bullet V_{skin} + AMT_{skin}}$$

Fat, kidney, and other non-metabolizing tissues:

Equation 10

$$\frac{dAMTtissuecap}{dt} = \frac{AMTart}{Vart} \bullet Qtissue + \frac{AMTtissue}{Vtissue} \bullet \frac{Qtissue}{Ptissue} \bullet Perm - \ldots$$
$$\frac{AMTtissuecap}{Vtissuecap} \bullet Qtissue - \frac{AMTtissuecap}{Vtissuecap} \bullet Qtissue \bullet Perm$$

Equation 11

$$\frac{dAMT_{tissue}}{dt} = \frac{AMT_{tissuecap}}{V_{tissuecap}} \bullet Q_{tissue} \bullet Perm - \frac{AMT_{tissue}}{V_{tissue}} \bullet \frac{Q_{tissue}}{P_{tissue}} \bullet Perm$$

Table 2. Parameters used in model simulations.

Parameter Symbol	Parameter Description	Parameter Value
Physiological Parameters		
BW	Body weight (kg)	Males-81.65 Females-65.77
CO	Cardiac output (L/hr/kg $^{0.7}$)	4.46
QPC	Ventilation rate (L/hour/kg $^{0.7}$)	8.43
VARC	Fraction arterial blood	0.0224
VVC	Fraction venous blood	0.0448
VALC	Fraction alveolar space	0.005
VLUC	Fraction lung tissue	0.014
VLIC	Fraction liver tissue	0.026
VFC	Fraction fat tissue	0.213
VKC	Fraction kidney tissue	0.0044
VSC	Fraction skin tissue	0.035
VOC	Fraction other tissue	0.6399
TCLU	Lung capillary volume (% of tissue volume)	18.0
TCLI	Liver capillary volume (% of tissue volume)	13.8
TCF	Fat capillary volume (% of tissue volume)	2.0
TCK	Kidney capillary volume (% of tissue volume)	16.0
TCS	Skin capillary volume (% of tissue volume)	4.5
TCO	Other capillary volume (% of tissue volume)	4.5
QLC	Fractional blood flow to Liver (% of cardiac output)	0.23
QFC	Fractional blood flow to Fat (% of cardiac output)	0.09
QKC	Fractional blood flow to Kidney (% of cardiac output)	0.175
QSC	Fractional blood flow to Skin (% of cardiac output)	0.058
QOC	Fractional blood flow to Other (% of cardiac output)	0.447
PERMF	Fat permeability	0.23
PERM	Capillary permeability	0.54
SA	Skin area (cm^2)	2000
Kp	Skin permeability (cm/hr)	10
Metabolic Parameters		
VMAXLI1	Capacity of Saturable Metabolism in Liver (mg/L/hr)	4.992×10^{-5}
KMLI1	Affinity of Saturable Metabolism in Liver (mg/L)	1.536×10^{-7}
VMAXLI2	Capacity of Saturable Metabolism in Liver (mg/L/hr)	7.273×10^{-6}
KMLI2	Affinity of Saturable Metabolism in Liver (mg/L)	1.984×10^{-7}
VMAXS1	Capacity of Saturable Metabolism in Skin (mg/L/hr)	4.992×10^{-5}
KMS1	Affinity of Saturable Metabolism in Skin (mg/L)	1.536×10^{-7}
n	Hill constant	2
VMAXLU	Capacity of Saturable Metabolism in Lung (mg/L/hr)	5.750×10^{-7}
KMLU	Affinity of Saturable Metabolism in Lung (mg/L)	1.536×10^{-7}
Chemical Parameters		
PB	Blood/Air Partition Coefficient	235.5
PLI	Liver/Blood Partition Coefficient	7.0
PLU	Lung/Blood Partition Coefficient	1.81
PF	Fat/Blood Partition Coefficient	160.4
PK	Kidney/Blood Partition Coefficient	4
PS	Skin/Blood Partition Coefficient	7.0
PO	Other/Blood Partition Coefficient	4
PERM	Capillary Permeability Constant	2.7
PERMF	Capillary Permeability Constant	1.2
Calculated Parameters		

QLI = QLC*CO	Liver Blood Flow (L/hr)
QF = QFC*CO;	Fat Blood Flow (L/hr)
QK = QKC*CO;	Kidney Blood Flow (L/hr)
QS = QSC*CO;	Skin Blood Flow (L/hr)
QO = QOC*CO;	Other Blood Flow (L/hr)
QTO = QLI+QF+QK+QS+QO;	Total Blood Flow (L/hr)
QV = QPC*BW$^{.74}$	Alveolar Ventilation (L/hr)
VAR = VARC*BW	Arterial blood volume (L)
VV = VVC*BW	Venous blood (L)
VAL = VALC*BW	Alveolar space (L)
VLU = VLUC*BW	Lung tissue (L)
VLI = VLIC*BW	Liver tissue (L)
VF = VFC*BW	Fat tissue (L)
VK = VKC*BW	Kidney tissue (L)
VS = VSC*BW	Skin tissue (L)
VO = VOC*BW	Other tissue (L)
VCLU = ((TCLU/100)*VLU)	Lung capillary volume (L)
VCLI = ((TCLI/100)*VLI)	Liver capillary volume (L)
VCF = ((TCF/100)*VF)	Fat capillary volume (L)
VCK = ((TCK/100)*VK)	Kidney capillary volume (L)
VCO = ((TCO/100)*VO);	Other tissue capillary volume (L)

Exposure scenarios:

We conducted simulations for different exposure scenarios based on the results of the passive naphthalene data (P. Egeghy , pers. com., Table 3). These exposures were developed from statistical models which adjusted the concentrations for the other variables in the model. This provides a population value for each base-sex combination, which should not be interpreted as an actual exposure for an individual worker. For this report, we compare a high exposure base with a low exposure base: for males, Davis Monthan and Seymour Johnson AFB, and for females, Davis Monthan and Pope AFB. We used an exponential distribution for Monte Carlo sampling of passive naphthalene concentrations and a normal distribution for skin contact concentrations.

Base	Sex	Mean	StDev
Davis Monthan AFB	M	758.40	987.02
Davis Monthan AFB	F	79.84	196.47
Hurlbert Field	M	347.82	395.28
Hurlbert Field	F	42.50	83.00
Langley AFB	M	134.24	206.00
Langley AFB	F	49.12	148.61
Little Rock AFB	M	134.54	233.38
Little Rock AFB	F	76.40	65.90
Pope AFB	M	240.60	336.40
Pope AFB	F	1.97	1.47
Seymour Johnson AFB	M	120.41	230.69
Seymour Johnson AFB	F	22.56	55.95

Table 3. Passive naphthalene concentration ($\mu g/m^3$) means and standard deviations by AFB and sex.

Findings: For males, the high exposure base was Davis Monthan. Predicted naphthalene in the various tissues is shown in Figures 2 and 3.

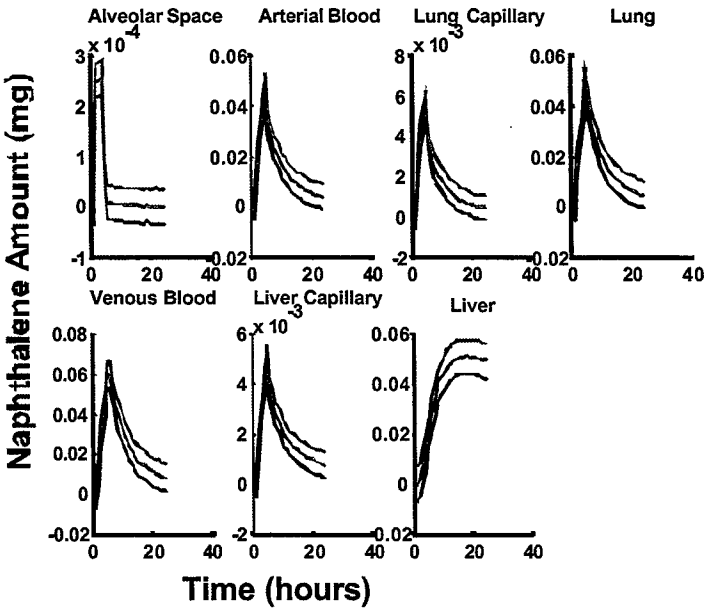

Figure 2. Predicted mean and 95% CI amounts (mg) of naphthalene in males at Davis Monthan AFB for 24 hours after an 4-hour exposure.

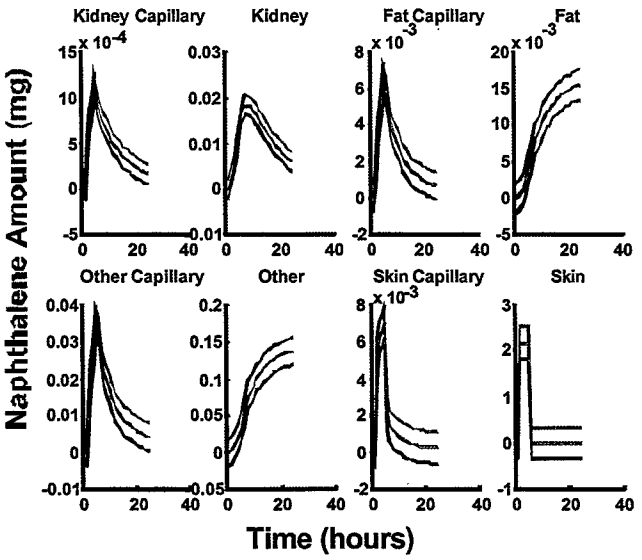

Figure 3. Predicted mean and 95% CI amounts (mg) of naphthalene in males at Davis Monthan AFB for 24 hours after an 4-hour exposure.

There is a rapid decrease in all compartments except the liver, fat, and other tissues following exposure. A simulation for a seven-day period with four-hour exposure for five days showed accumulation in the liver during the five days of exposure, but complete depletion after the two days of non-exposure. The accumulation in fat and other tissues likely is a result of the high fat:blood and tissue:blood partition coefficients.

We simulated exhaled breath to compare with measured results. We first compared males and females at Davis Monthan AFB (Figure 4).

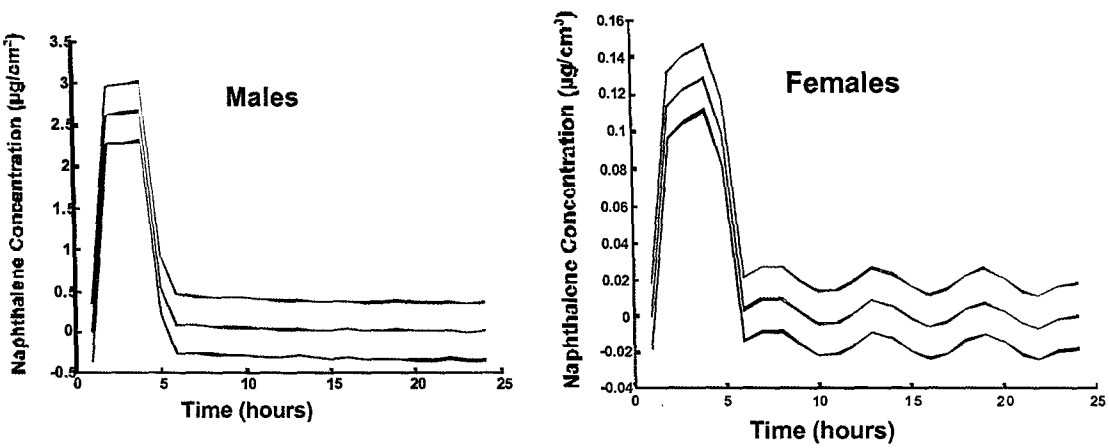

Figure 4. Predicted mean and 95% CI for naphthalene concentration ($\mu g/m^3$) in exhaled breath over 24 hours following a four-hour exposure in males (left) and females (right) at Davis Monthan AFB.

As in the other compartments, the predicted breath concentration increases rapidly after initial exposure and then drops rapidly after exposure. The predicted male concentration exceeded the female concentration by a factor of about twenty, whereas the measured male concentrations were about double the female. The predicted results underestimate the mean measured concentrations. For males, the mean measured concentration was (3.04±3.68) which was slight greater than predicted. For females, the measured mean concentration was 1.42±1.80, which exceeded the predicted value by about a factor of ten. Oscillations seen in the females result from low uptake and redistribution of naphthalene among various tissues.

We compared Bases for males (Figure 5) and females (Figure 6).

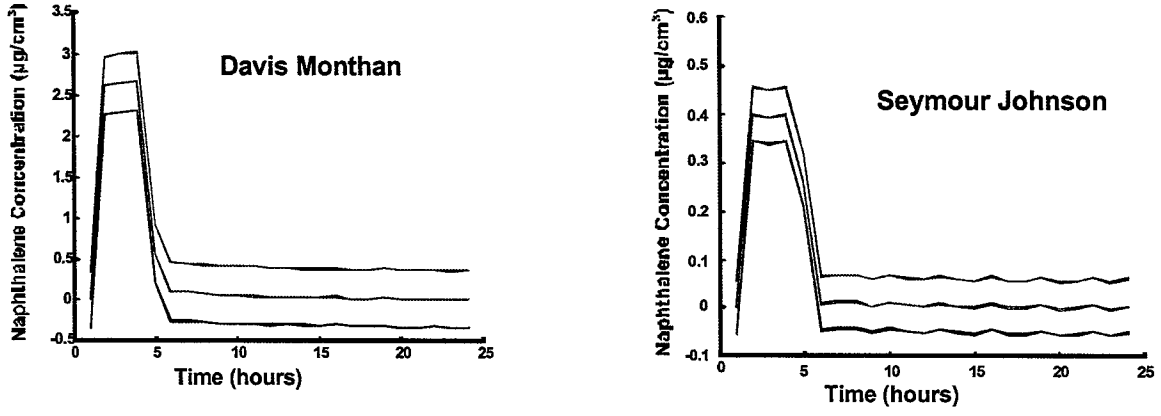

Figure 5. Predicted mean and 95% CI for naphthalene concentration (µg/m^3) in exhaled breath over 24 hours following an eight-hour exposure in males at Davis Monthan AFB (left) and Seymour Johnson AFB (right).

Predicted peak mean male concentrations at Seymour Johnson AFB, the lowest of any AFB, were about 0.4 µg/m^3 compared with measured mean concentrations of 1.23 µg/m^3. This represents an underestimate of a factor of about three. At both bases, the predicted naphthalene concentrations reach their peak within the first hour and then decline to near zero within two hours after exposure ends.

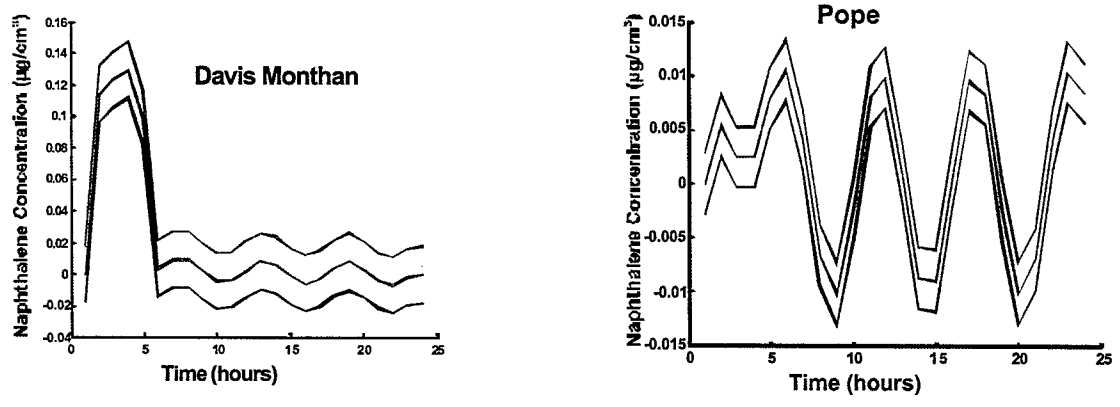

Figure 6. Predicted mean and 95% CI for naphthalene concentration (µg/m^3) in exhaled breath over 24 hours following an eight-hour exposure in females at Davis Monthan AFB (left) and Pope AFB (right).

Predicted peak mean female concentrations at Pope AFB, the lowest of any AFB, were about 0.01 µg/m^3 compared with measured mean concentrations of 1.01µg/m^3. This represents an underestimate of a factor of about a hundred. Because of the low ambient concentrations (1.97±1.47 µg/m^3), there is not the rapid rise in exhaled breath concentrations seen at other bases; there are low oscillations seen in the other figures after depuration.

Discussion and Conclusion

The model predictions of the naphthalene concentrations in exhaled breath compare favorably with measured values at the highest exposure concentrations. Model predictions, however, significantly underestimate exhaled breath concentrations of naphthalene at low exposures (primarily in female workers). There appears to be a minimum naphthalene concentration in post-exposure breath concentrations of around 1.0–1.5 $\mu g/m^3$. This minimum could result from long-term chronic exposure to low concentrations. We simulated only acute exposures so if there is significant chronic exposure, that could account for some of the differences between observed and predicted breath concentrations. We were not able to compare other compartments such as blood or urine, as these data were not available as of this writing.

There is much uncertainty in the model. Actual exposure concentrations, particularly for dermal exposure, could be improved (e.g., level and duration of exposure for different job classes). The measured dermal data were not available for this report. We also need better parameter estimates, especially naphthalene-specific parameters such as the partitioning coefficients. All of the current naphthalene PBPK models were developed for mice and rats and did not include human parameters. In the DHHS model, metabolic rates (for mice and rats) were estimated by optimizing the model to naphthalene blood time course data. Such an approach should be possible with the data from this study. The partitioning coefficients in all of the models were estimated from the log octanol:water PC and oil:air and water:air PCs. A similar approach could provide similar PCs for humans. As pointed out by Quick and Shuler, "Experiments need to be conducted to determine whether naphthalene and naphthalene oxide transport are diffusion- or flow-limited. In either case, direct measurement of the parameters describing the transport (diffusion rates or tissue:blood PCs) are needed."

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A Statistical Analysis of Risk and Exposure Data Collected for the Risk Assessment of Acute Exposure to Jet Fuel Study

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Abstract

Linear mixed models are fit to SL (Sway Length) and SA (Sway Area) from the postural sway tests and to two of the variables (Match-to-sample and Tapping) from the GASH/BARS tests. After reducing the number of variables in the models (without significantly affecting the fit of the models), a "best case/worst case" approach is used to determine what the worst possible effect would be when non-zero levels of naphthalene in the breath are observed. The analysis determines what performance would be expected from an individual with no naphthalene present, and then compares that performance to what would be expected from the same individual with non-zero levels of naphthalene in the breath, assuming the worst case.

Contents

Glossary

Covariate : A variable which may (or may not) have an effect upon the response variable.

Interaction : A phenomenon where a variable behaves differently at different levels of one or more other variables. For example, consider two factors A and B, each measured at a high and low level. Suppose there is a decrease in the response as A moves from low to high while B is at the low level, and there is an increase in the response as A moves from low to high while B is at the high level. The behavior of the response as A changes is different depending on what level B is at. This is an interaction.

P-value : A measure of the amount of evidence to support claiming that there is a significant relationship. P-values below 0.05 are commonly accepted as indicating a significant relationship. For this analysis, p-values between 0.05 and 0.10 will be considered marginally significant.

Significant : To say that there is a significant relationship means that the data support the claim of an actual relationship. To claim that there exists a significant relationship does not mean that the relationship actually exists. Further, to claim that there is no significant relationship does not mean that the relationship does not actually exist.

Response Variable : The variable of interest in the study. In this study, these are the performance and health measurements taken on each subject.

Data Dictionary

ACTIVITY : An identifier indicating whether or not the subject had any unusually stressful activity in the last 24 hours. Values: 1 = Yes, 2 = No

AFQTADMN, AFQTELCT, AFQTGEN, AFQTMECH : Armed Forces Qualification Test scores on entry to military service. Values (each): 0-100

AGE : Age in years of the subject.

AGE_DAYS : Age in days of the subject. Computed as DOT-DOB.

ALCOHOL : An identifier indicating whether or not the subject drinks alcoholic beverages. Values: 1 = Yes, 2 = No, 3 = Choose not to answer.

ASLEEP : Time the subject went to sleep the night before testing.

AVGLEN : The average length of the subject's feet in cm. Computed as (R_LENTH + L_LENTH)/2

AVGWID : The average width of the subject's feet in cm. Computed as (R_WIDTH + L_WIDTH)/2.

AWAKE : Time the subject awoke the day of testing.

BASE ID : The base at which the subject was stationed during the study. These bases are abbreviated DAV (Davis Monthan AFB), HUR (Hurlburt Field, FL), LAN (Langley AFB), LIT (Little Rock AFB), POP (Pope AFB), and SEY (Seymour Johnson AFB).

BMI : Body mass index. Defined as $W/H^2 \times 703$, where H is the height in inches and W is the weight in pounds.

BRTH_N : Naphthalene concentration in exhaled breath (in $\mu g/m^3$). Defined as PRE_N if SESSION = "Pre" and POST_N if SESSION = "Post".

CATEGORY : The degree of exposure to JP8 of the subject. Values: "LOW", "MOD", "HI"

DOB : Date of birth.

DOT : Date of testing.

EAR_INF : An identifier indicating whether or not the subject currently has an ear infection. Values: 1 = Yes, 2 = No

EFSCORE : SF-36 psychological factor. Values: 0-100

EMSCORE : SF-36 psychological factor. Values: 0-100

EYES : An identifier indicating whether or not the subject's eyes were open or closed during the postural sway tests. Values: "Open", "Closed"

FALLEN : An identifier indicating whether or not the subject has fallen on the job in the last 12 months. 1 = Yes, 2 = No

GENDER : Gender of the subject. Values: 1 = Male, 2 = Female

GHCHSCRE : SF-36 psychological factor. Values: 0-100

GHPSCORE : SF-36 psychological factor. Values: 0-100

HEIGHT : Height of the subject in inches.

HISP : An identifier indicating whether or not the subject is Hispanic. Values: 1 = Yes, 2 = No

HRS_AWK : Hours since the subject awakened. Computed as TST_TIME – AWAKE.

HRS_MENT_WRK : Hours spent performing mental work in past 12 hours. Computed as HRS_WRK × MENT_WRK.

HRS_PHYS_WRK : Hours spent performing physical work in past 12 hours. Computed as HRS_WRK × PHYS_WRK.

HRS_SLPT : Hours the subject slept the night before.

HRS_WRK : The hours the subject has worked in the last 12 hours.

L_LENTH : Length of the left foot measured at the time of the postural sway test. Measured in cm.

L_WIDTH : Width of the left foot measured at the time of the postural sway test. Measured in cm.

MEAL : Hours since the subject last ate a meal

MENT_WRK : Percent of time (of HRS_WRK) spent performing mental work.

MENTAL : A numeric measure of the amount of mental stress the subject is under. Values: 0-9, 0 = Least Stress, 9 = Most Stress

MHSCORE : SF-36 psychological factor. Values: 0-100

MS_SC : Match-to-sample composite score. Sum of MS_SC1, MS_SC2, and MS_SC3, for which there was a 1 second, 8 second, and 16 second delay, respectively. Values: 0-45

MTHBASE : Months stationed at current base.

MTHJOB : Number of months subject has worked at current job.

PAINSCRE : SF-36 psychological factor. Values: 0-100

PASS_N : Breathing zone naphthalene exposure (in $\mu g/m^3$) measured by a passive monitor.

PEXERT : A numeric measure of the amount of physical stress the subject is under. Values: 0-24, 0 = Least Stress, 24 = Greatest Stress

PFSCORE : SF-36 psychological factor. Values: 0-100

PHYS_WRK : Percent of time (of HRS_WRK) spent performing physical work.

PHYSNTWK : The amount of physical work required for non job-related activities. Values: 1 = A great deal, 2 = A moderate amount, 3 = A little, 4 = None, 5 = Choose not to answer

PHYSCORE : SF-36 psychological factor. Values: 0-100

PHYSWRK : The amount of physical work required on the subject's job. Values: 1 = A great deal, 2 = A moderate amount, 3 = A little, 4 = None, 5 = Choose not to answer

POST_N : Naphthalene concentration in exhaled breath (in $\mu g/m^3$) after performing assigned tasks.

PRE_N : Naphthalene concentration in exhaled breath (in $\mu g/m^3$) before performing assigned tasks.

R_LENTH : Length of the right foot measured at the time of the postural sway test. Measured in cm.

R_WIDTH : Width of the right foot measured at the time of the postural sway test. Measured in cm.

RACE, RACE2 : An identifier indicating subject's race. Values: 1 = White (European), 2 = Black (African-American), 3 = Native American, 4 = Asian/Pacific Islander, 5 = Other

SA : The total sway area traveled by the center of pressure for the postural sway tests. Measured in cm^2.

SBMI : Body mass index computed from SHEIGHT and SWEIGHT.

SESSION : The session at which the postural sway test was conducted. Values: "Pre", "Post"

SFSCORE : SF-36 psychological factor. Values: 0-100

SHEIGHT : The height measured at the time of the postural sway test. Measured in inches.

SL : The total sway length traveled by the center of pressure for the postural sway tests. Measured in cm.

SLP_INTR : An identifier indicating whether the subject's sleep was interrupted the night before. Values: 1 = Yes, 2 = No, 3 = Unknown

SMOKER : An identifier indicating the amount the subject smokes. Values: 1 = 21+ cigarettes per day (1+ pack), 2 = 11-20 cigarettes per day (1 pack), 3 = 6-10 cigarettes per day (1/2 pack), 4 = 1-5 cigarettes per day (1/4 pack), 5 = Do not smoke, 6 = Choose not to answer

STRESS : An identifier indicating whether the subject had any stressful events at home or work in the last 24 hours. Values: 1 = Yes, 2 = No

SUBID : The unique identifier assigned to each subject. The identifier consists of the first three letters of the base the subject is stationed at plus a 4 digit number.

SURFACE : The surface the subject was standing on during the postural sway tests. Values: "Plate" – Subject standing on the plate upright, "Plate/Bending" – Subject standing on the plate while bending over, "4" Foam" – Subject standing on a foam pad upright

SWEIGHT : Weight measured at the time of the postural sway test. Measured in pounds.

TAPP : Number of taps on a button made by the subject with their preferred hand. This variable is a combination of TAPP1, and TAPP2, which are the two repetitions of tapping with the preferred hand. Values: 0-250

TST_TIME : The time of day of the postural sway test.

WEIGHT : Weight of the subject in pounds.

WORK : An identifier indicating whether the subject had to work in the last 24 hours. Values: 1 = Yes, 2 = No

Chapter 1
Performance and Balance Measurements

1.1 Introduction

The group from the University of Cincinnati headed by Dr. Amit Bhattacharya collected postural balance measurements for 136 of the subjects involved in the study. Table 1.1 lists the breakdown by base and the dates during which the tests were conducted. Each subject was given a questionnaire prior to testing from which the values of the variables DOT, ASLEEP, AWAKE, HRS_SLPT, SLP_INTR, MEAL, EAR_INF, FALLEN, ACTIVITY, STRESS, WORK, HRS_WRK, PHYS_WRK, and MENT_WRK were obtained. The variables SWEIGHT, SHEIGHT, SL, SA, TST_TIME, R_LENTH, R_WIDTH, L_LENTH, and L_WIDTH were also obtained at this time. Prior to performing his or her assigned tasks, each subject underwent a battery of tests. These tests consisted of different combinations of keeping their eyes open or closed, standing on different surfaces, as well as bending over during the test.

It is of interest to determine the extent to which exposure to JP8 has an effect on a subject's performance in these tests. To do this, a model was built to relate the two sway measurements (SL and SA) to the various covariates using the SAS system.

Base	Subjects Tested	Dates
Davis Monthan	26	April 10-14, 2000
Hurlburt Field	15	September 18-21, 2000
Langley	23	June 12-16, 2000
Little Rock	24	August 21-25, 2000
Pope	25	July 17-21, 2000
Seymour Johnson	23	May 15-19, 2000

Table 1.1: Numbers of Subjects Examined

1.2 The Data

Each subject was tested a total of 20 times. Ten of these tests were conducted prior to the subject performing his or her assigned tasks, and ten were conducted after the subject performed his or her assigned tasks. During each of these time periods, the subjects were tested with their eyes open or closed and under three different surface/posture combinations. Table 1.2 lists these combinations and the number of replications for each combination.

Of the 136 subjects measured by Dr. Bhattacharya's group, three were completely removed from the data set (DAV3367, DAV3553, DAV4858) due to concerns raised at the 4/13 meeting in Cincinnati of cross-contamination. Of the remaining 133 subjects, seven have at least one covariate with missing values. These are outlined in Table 1.3. It should be noted that these subjects are only removed from the analysis if the variables that have missing values are included in the model. Notice that only 9 subjects are listed in Table 1.3. Subject LIT1512 is missing only L_WIDTH from the "Post" SESSION. The value of AVGWID (which is used in the analysis instead of both L_WIDTH and R_WIDTH, as explained below) is set to be

R_WIDTH only for this subject. As there were a total of 20 tests performed for each subject, the maximum possible number of observations is 2720. In this analysis, however, 2580 of these observations are actually used to build the model.

Session	Eyes	Surface/Posture	Number of Replications
Pre	Open	Plate/Upright	2
Pre	Open	Plate/Bending	1
Pre	Open	4" Foam/Upright	2
Pre	Closed	Plate/Upright	2
Pre	Closed	Plate/Bending	1
Pre	Closed	4" Foam/Upright	2
Post	Open	Plate/Upright	2
Post	Open	Plate/Bending	1
Post	Open	4" Foam/Upright	2
Post	Closed	Plate/Upright	2
Post	Closed	Plate/Bending	1
Post	Closed	4" Foam/Upright	2
			Total: 20

Table 1.2: Summary of Sway Tests

Subject	Observations Affected	Reasons/Variables Missing
DAV3367	20	Possible cross-contamination
DAV3553	20	Possible cross-contamination
DAV4858	20	Possible cross-contamination
HUR8712	20	SMOKER and ALCOHOL missing
LIT2851	10	MEAL missing from "Post" SESSION
LIT3340	10	MEAL missing from "Post" SESSION
LIT4209	20	MEAL missing
LIT4560	10	ACTIVITY, STRESS, MEAL, HRS_SLPT, HPHYSWRK, HMENTWRK, and HRSAWK all missing from "Post" SESSION
LIT5278	10	MEAL missing from "Post" SESSION
	Total: 140	

Table 1.3: Summary of Missing Observations

1.3 Variance-Covariance Structure

In many experiments, each observation collected is independent of every other observation collected. In this particular experiment, however, this is not the case. Knowing, for example, that a subject performed extremely well during one of the tests, it would be reasonable to expect that the subject would perform well for the other tests. That is, the observations within a subject are correlated. In addition to building an appropriate model to relate SL and SA with the other covariates, it is also necessary to find an appropriate variance-covariance structure. That is, the way in which the observations within a subject are correlated with one another must be described. This is accomplished by specifying the variance-covariance matrix, Σ. As a simple illustration of a variance-covariance matrix, consider three random variables denoted X, Y, and Z. The variance-covariance matrix for the three random variables may be written as

$$\Sigma = \begin{matrix} \begin{bmatrix} \sigma_X^2 & \sigma_{XY} & \sigma_{XZ} \\ \sigma_{XY} & \sigma_Y^2 & \sigma_{YZ} \\ \sigma_{XZ} & \sigma_{YZ} & \sigma_Z^2 \end{bmatrix} \begin{matrix} X \\ Y \\ Z \end{matrix} \\ \begin{matrix} X & Y & Z \end{matrix} \end{matrix}$$

Each diagonal element is the variance for the associated random variable, and each off-diagonal element is the covariance between the two associated random variables. In this experiment, there are 20 such variables (the random response from each of the 20 tests), and the variance-covariance matrix is 20 by 20. Since the matrix must be symmetric, there are a total of 210 variance and covariance parameters that may be allowed to vary. The most general case (hereafter known as the *unstructured model*) is that no structure is imposed on the matrix, and each of the 210 parameters is allowed to vary independent of every other parameter. This has the advantage that it is completely general, but it has drawbacks as well. Consider the following from Lindsey (1999):

> *In some cases, such generality is necessary, when the stochastic dependence relationships among the responses are not known to have any specific structure from theoretical considerations or previous empirical research. It might also be justified as a sort of semi-parametric model, when one is only interested in the mean relationships. Although this may have the advantage of a minimum of hypotheses, it usually involves reduced efficiency and validity in inferences about the parameters of interest (Altham, 1984), as well as often being less informative about underlying mechanisms producing the data.*

Because of these concerns, several different covariance structures are examined and compared to the unstructured model. Three different criteria are used for this comparison. The first is a chi-square test to determine if the unstructured model is a significant improvement over the reduced covariance structures. Let L_R be the likelihood function for the reduced model evaluated at the maximum likelihood estimates (MLEs), and let L_F be the likelihood for the full model. It can be shown (under mild regularity conditions) that $2[\ln(L_F) - \ln(L_R)]$ has an approximate chi-square distribution with degrees of freedom given as the number of free variance-covariance parameters for the full model minus the number of free variance-covariance parameters for the reduced model. The second criterion is known as Akaike's Information Criterion (AIC). It is defined as $\ln(L) - q$, where q is the number of free variance-covariance

parameters. Keselman et al. (1998) show through a simulation study that using the AIC criterion can result in choosing the incorrect variance-covariance structure a significant proportion of the time even if the correct variance-covariance structure is one of those under consideration. Their work was done assuming a very basic repeated measures design. Finally, the third, and least important, criterion is the value of R^2 for the model, computed in the usual manner.

One of the simplest variance-covariance structures for which the off-diagonals of Σ are non-zero is the *compound symmetric* structure. The diagonals (variances) are defined to be σ^2, while the off-diagonals are all defined to be $\sigma^2 + \sigma_1$. That is, the covariance between any pair of the random variables is the same as any other pair. This model will serve as a baseline in the search for the "best" model.

It seems reasonable to assume that two tests that are performed under the same conditions would be more highly correlated than two tests that are performed under much different conditions. Two ways of modeling this hypothesized behavior are considered. The diagonals are again defined to be σ^2. The value of each off-diagonal is defined to be σ_i, where i equals the number of covariates (of EYES, SURFACE, and SESSION) that have different values for the two tests under consideration. For example, let the first test be with EYES = "Open", SURFACE = "Plate", and SESSION = "Pre", while the second test has EYES = "Open", SURFACE = "Plate/Bending", and SESSION = "Post". Then the covariance between these two tests is defined to be σ_2 since two of the variables (SURFACE and SESSION) have different values. This yields a total of 5 variance-covariance parameters to estimate, σ^2, and σ_0 through σ_3. If intuition is correct, and the tests that are most similar are more highly correlated, then it would be expected that $\sigma_3 < \sigma_2 < \sigma_1 < \sigma_0$. For the purposes of this analysis, this covariance structure will be known as the *difference model*. This model can easily be made more general by allowing each of the 20 variables to have a different variance. This will be referred to as the *nonhomogeneous difference model*.

The second method of modeling the hypothesized behavior is to again define the diagonals to be σ^2 while the off-diagonals are defined to be

$$\begin{aligned}\sigma_0 &+ \sigma_1 I(\text{EYES} : \text{"Open"} \leftrightarrow \text{"Closed"}) \\ &+ \sigma_2 I(\text{SURFACE} : \text{"Plate"} \leftrightarrow \text{"4" Foam"}) \\ &+ \sigma_3 I(\text{SURFACE} : \text{"Plate"} \leftrightarrow \text{"Plate/Bending"}) \\ &+ \sigma_4 I(\text{SURFACE} : \text{"4" Foam"} \leftrightarrow \text{"Plate/Bending"}) \\ &+ \sigma_5 I(\text{SESSION} : \text{"Pre"} \leftrightarrow \text{"Post"}),\end{aligned}$$

where I(VAR : "Val 1" $\leftrightarrow$ "Val 2") is defined to be 1 if the value of the variable VAR changes from "Val 1" to "Val 2" or vice versa, and is defined to be 0 otherwise. Thus, σ_0 is the covariance between two tests that are performed under the same conditions. If there are any differences, the type and number of differences will have a cumulative effect on the value of the covariance. Using the example from the preceding paragraph, the covariance between the two tests would be $\sigma_0 + \sigma_3 + \sigma_5$. This structure has 7 variance-covariance parameters to estimate, σ^2 and σ_0 through σ_5. Furthermore, the value of σ_0 should be positive, while the values of σ_1 through σ_5 should be negative, representing a reduction in the covariance for tests that are

different. This covariance structure will be known as the *additive model.* Similar to the difference model, this model can also be made more general. This will be known as the *nonhomogeneous additive model.*

1.4 Model Selection

Linear models were fit to ln(SA) and 2*ln(SL)-6.5 (The transformations were needed to satisfy the usual assumptions placed on the residuals.) for the following covariates: BASEID, EYES, SURFACE, EYES×SURFACE interaction, SESSION, BRTH_N (defined as the value of PRE_N if SESSION = "Pre" and POST_N otherwise) GENDER, FALLEN, SMOKER, ALCOHOL, ACTIVITY, STRESS, SWEIGHT, SHEIGHT, SBMI, AVGWID, AVGLEN, HRS_SLPT, MEAL, HPHYSWRK, HMENTWRK, AGEDAYS, MTHBASE, MTHJOB, and HRSAWK. The full models (including all covariates) were run assuming each of the variance-covariance structures using PROC MIXED in SAS. For each, insignificant variables were removed one at a time to yield a simplified model. The variable BRTH_N was retained regardless of its significance since it is of interest to study the magnitude of the effect of BRTH_N on SA and SL. In each case, the reduced model had a value of R^2 that was reduced less than 1½% as compared to the R^2 value for the full model.

Due to limitations in the measurement process, values of naphthalene concentration below ½ $\mu g/m^3$ were unobservable. Of the 333 total subjects included in the "Effects Group" master Excel spreadsheet, 195 had a value of PRE_N below the observable limit, while 76 had a value of POST_N below the observable limit. Because of the extremely large number of subjects this affects, a "best case/worst case" approach will be taken in the analysis. One analysis substitutes all values of BRTH_N below the observable limit with 0 $\mu g/m^3$, while the other analysis substitutes all values of BRTH_N below the observable limit with ½ $\mu g/m^3$. The analysis that shows the effect of the variable BRTH_N on the values of SA and SL to be the largest is adopted, since it represents the worst case that would be expected given the data and the models applied to the data.

The results for the final models chosen are summarized in Tables 1.4 and 1.5. The results for the difference and additive models are excluded since the nonhomogeneous versions of both models provide slightly to significantly better fits. Further, the results for the compound symmetric and nonhomogenous additive models are excluded, as the value of AIC is much lower than for the unstructured and nonhomogenous difference models. Although the chi-square test is highly significant for the nonhomogeneous difference model, it cannot be entirely ruled out because the value of AIC is comparable to that for the unstructured model (although slightly lower) and it is a much more parsimonious model than is the unstructured model. It is also unknown what effect a misspecification of the variance-covariance structure has on the validity of the F-tests reported by SAS's PROC MIXED. Further, because of the statement of Lindsey (1999) given above and the results of Keselman et al. (1998), the analysis proceeds by considering both models. The entries in the cells of Tables 1.4 and 1.5 are the p-values (based on Type III sums of squares) to test for the significance of the listed effect. Only those variables that remain in the model are listed. Those with a p-value at or below 5% are considered statistically significant, while those with a p-value between 5-10% are considered marginally significant for the purposes of this analysis. At the bottom of the table, several summary statistics for the model are listed. In addition, the estimated coefficient for BRTH_N is supplied,

as well as the upper end of the 95% confidence interval for its true value. It is worth noting that the p-value for BRTH_N is, under every model, well above the 5% significance level.

ln(SA)	Covariance Structure			
Effect	Unstructured (0)	Nonhomogeneous Difference (0)	Unstructured (0.5)	Nonhomogeneous Difference (0.5)
BASEID	0.0003	0.0053	0.0003	0.0070
EYES	<0.0001	<0.0001	<0.0001	<0.0001
SURFACE	<0.0001	<0.0001	<0.0001	<0.0001
EYES×SURFACE	<0.0001	<0.0001	<0.0001	<0.0001
BRTH_N	0.6391	0.3501	0.8772	0.7706
GENDER	0.0264		0.0327	0.0933
SMOKER	0.0035		0.0035	
SWEIGHT		0.0003		<0.0001
SBMI		0.0059		0.0009
AVGWID	0.0106	0.0011	0.0052	0.0054
AVGLEN	<0.0001		<0.0001	
MTHBASE		0.0574		0.0634
MTHJOB		0.0177		0.0125
Log Likelihood	-726.8	-938.9	-719.6	-924.5
Akaike	-936.8	-962.1	-929.6	-948.5
Var Params	210	24	210	24
R^2	0.65943	0.67997	0.65921	0.68236
BRTH_N Coefficient (Upper 95% CL)	-0.00262 0.00832	-0.00893 0.00979	-0.00064 0.00750	-0.00210 0.01204

Table 1.4: Summary of PROC MIXED for ln(SA). (Note: The number in parenthesis is the value of BRTH_N used if it is actually below the observable limit. Effects with empty cells were not included in the model.)

1.5 Model Validity

The assumption placed on the model developed is that the residuals of the model are jointly normally distributed with a constant variance-covariance structure. To test this assumption, a test of normality is conducted for each set of residuals from each unique testing situation, giving a total of 20 sets. Under the assumption given above, each group of residuals should form a normal random sample with a constant variance. The usual tests of normality are then applied to each group. For the analysis on SA, two of the sets of residuals have test statistics that are consistently significant at the 5% level (among the four tests SAS performs), while the other 18 fail to consistently reject the null hypothesis of normality. This is not a surprising result as it has a 19% chance of occurring with data that are truly normal. Furthermore, none of the tests are significant after a Bonferroni correction is applied. For the analysis on SL, none of the groups have test statistics that are consistently significant. To assess the appropriateness of the constant variance assumption, no formal tests are applied, but as is typical, residual versus fitted plots are

produced for each group. For both SA and SL, no plots suggest any deviation from this assumption. Furthermore, no plots show any irregular patterns that would suggest a lack-of-fit of the model.

2*ln(SL)-6.5	Covariance Structure			
Effect	Unstructured (0)	Nonhomogeneous Difference (0)	Unstructured (0.5)	Nonhomogeneous Difference (0.5)
BASEID	0.0002	0.0329	0.0007	0.0323
EYES	<0.0001	<0.0001	<0.0001	<0.0001
SURFACE	<0.0001	<0.0001	<0.0001	<0.0001
EYExSURFACE	<0.0001	<0.0001	<0.0001	<0.0001
BRTH_N	0.7944	0.5941	0.8553	0.5697
SWEIGHT	0.0578		0.0669	
SHEIGHT		0.0040		0.0047
AVGWID		0.0155		0.0181
AVGLEN	0.0398			
HRS_SLPT		0.0694	0.0863	0.0955
MTHBASE		0.0301	0.0426	0.0320
HRSAWK		0.0526	0.0085	0.0628
Log Likelihood	-203.7	-406.8	-189.7	-401.1
Akaike	-413.7	-430.8	-399.7	-425.1
Var Params	210	24	210	24
R^2	0.68559	0.71508	0.69294	0.71226
BRTH_N Coefficient (Upper 95% CL)	0.00145 0.01236	-0.00494 0.01324	0.00076 0.00886	-0.00409 0.00999

Table 1.5: Summary of PROC MIXED for ln(SL). (Note: The number in parenthesis is the value of BRTH_N used if it is actually below the observable limit. Effects with empty cells were not included in the model.)

1.6 Effects of BRTH_N on SA and SL

With the models built, it is clear that the "worst case," here being defined as that model with the largest possible effect for BRTH_N, is, for SA, the nonhomogeneous difference model where ½ $\mu g/m^3$ is used for the value of BRTH_N when it is below the detectable limit. For SL, the nonhomogenous difference model where 0 $\mu g/m^3$ is used for the value of BRTH_N when it is below the detectable limit is the "worst case" model.

Two hypothetical subjects were created, and the chosen "worst case" models are applied to each subject. The characteristics of the hypothetical subjects, one female and one male, are given in Table 1.6. The values are chosen to reflect an "average" subject that participated in the study. One of each of these hypothetical subjects is then added to the data set for every base, and predicted values of SA and SL (actually, log(SA) and 2*log(SL)-6.5) are obtained for values of

BRTH_N ranging from 0 $\mu g/m^3$ to 16 $\mu g/m^3$. (It is important to note that adding these hypothetical subjects to the data set in no way affected the model building procedure, as the values of the response variable are entered as missing data.) The higher value is chosen to be 16 $\mu g/m^3$ because the largest observed breath measurement in the data set is 15.8 $\mu g/m^3$. (The largest such measurement in the entire group of 332 subjects is 36.3 $\mu g/m^3$ for PRE_N. That subject was not included in the postural sway study, however.) These predicted values are computed by SAS using the predicted coefficient associated with BRTH_N, which does not represent the "worst case". Thus, the predicted values were manually adjusted using the upper limit of the 95% confidence intervals given in Tables 1.4 and 1.5.

Variable	Male Subject	Female Subject
SWEIGHT (pounds)	180	145
SHEIGHT (inches)	70	65
AVGLEN (cm)	26.5	24
AVGWID (cm)	10	9
MTHBASE (months)	36	36
MTHJOB (months)	56	56
HRSAWK (hours)	3 Pre, 10 Post	3 Pre, 10 Post
HRSSLPT (hours)	6.5	6.5
EYES	Open	Open
SURFACE	Plate	Plate

Table 1.6: Hypothetical subjects constructed for analysis.

For each of the combinations of GENDER, BASEID, and SESSION, the predicted value when BRTH_N is equal to zero represents the case when the subject has no (measurable) naphthalene present in his or her breath. For each of these cases, if the normal distribution is assumed for the model (and there is no evidence to support assuming otherwise), the predicted value and its associated standard error for prediction define the predicted (normal) distribution of SL and SA for such a subject. Thus, they can be used to supply a range of "typical" values of SL and SA that one would expect from such a subject. For this analysis, the 95^{th} percentile of the estimated distributions for SA and SL are computed. That is, the value of SA and SL for which 95% of the observations for similar subjects would be beneath. This is taken to be a "critical value" of sorts. Any observation above this value happens rarely enough that if it were to be observed to happen, it would be more likely due to something other than random chance.

Once these 95^{th} percentiles are computed, the estimated probability of exceeding the percentile is computed for each value of BRTH_N up to 16 $\mu g/m^3$. This probability gives a measure of the severity of the effect that BRTH_N has on SA and SL, in the worst possible case. As an example, consider the male subject from Davis Monthan AFB when SESSION = "Pre". The predicted value of SA when BRTH_N = 0 $\mu g/m^3$ is 0.93592 (in the log scale, which translates to 2.55 cm^2) with a standard error of prediction equal to 0.44853. The 95^{th} percentile for this distribution is 1.67369 (5.33 cm^2 in the original scale). The probability of the same subject with a value of BRTH_N equal to 16 $\mu g/m^3$ exceeding 1.67369 is, in the worst possible case, equal to 11.9%, which represents an increase of only 6.9%. Figure 1.1 plots these

probabilities for SA for all possible combinations of BASEID, SESSION, and GENDER, yielding 24 different lines. Because they are all extremely similar, they are all plotted on the same graph. The conclusions reached for a hypothetical subject in one situation would be virtually identical for the same subject in another situation (different BASEID and/or SESSION). Figure 1.2 plots the probabilities for SL. The 95th percentiles (in the original scale) used to compute the probabilities in Figures 1.1 and 1.2 are given in Tables 1.7 and 1.8. Note that the values for BASEID = "LAN" are well below the percentiles for the other 5 bases. This difference is not explained by any of the variables included in the model, and is certainly something that should be studied further to determine what the reason for this is.

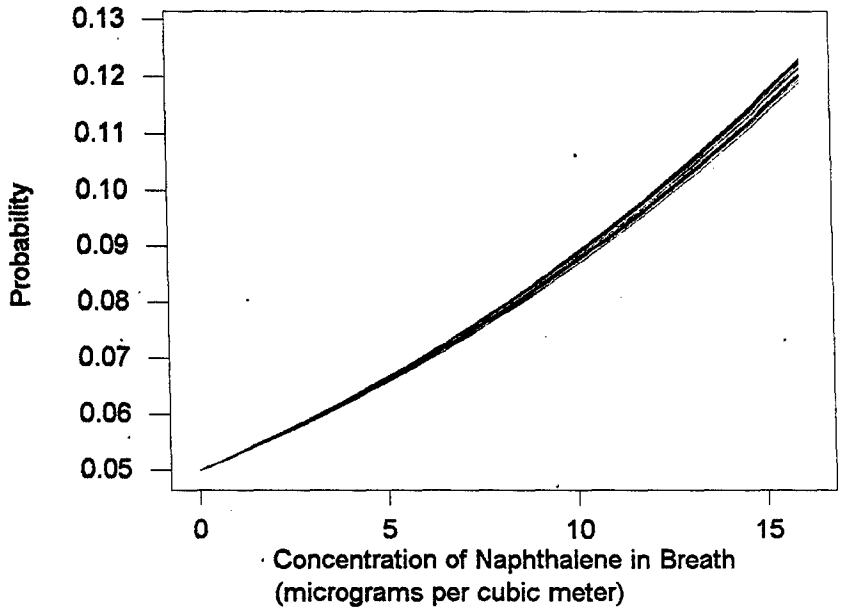

Figure 1.1. Probability of exceeding 95th percentile of SA as a function of BRTH_N.

1.7 Conclusions

Mixed models were constructed to predict for the variables SA and SL based on the covariates BASEID, EYES, SURFACE, EYES×SURFACE interaction, SESSION, BRTH_N, GENDER, FALLEN, SMOKER, ALCOHOL, ACTIVITY, STRESS, SWEIGHT, SHEIGHT, SBMI, AVGWID, AVGLEN, HRS_SLPT, MEAL, HPHYSWRK, HMENTWRK, AGEDAYS, MTHBASE, MTHJOB, and HRSAWK. A total of four models were fit to each of SL and SA, depending on how values of BRTH_N were handled and the type of variance-covariance matrix

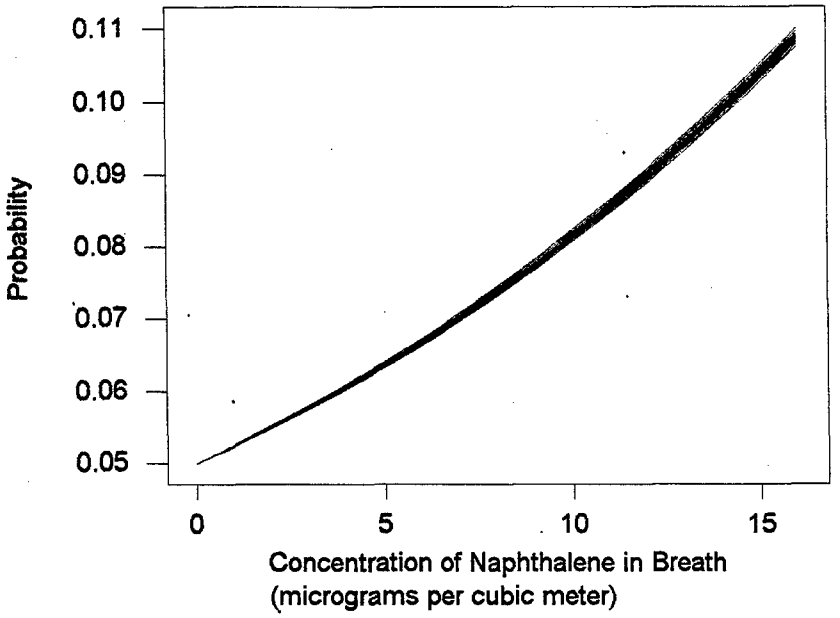

Figure 1.2. Probability of exceeding 95th percentile of SL as a function of BRTH_N.

used. The insignificant covariates were removed one at a time to yield reduced models for SA and SL. The reduction in the fit was minimal, so little was lost in removing the extraneous covariates. In both cases, the model that cast the effect of BRTH_N in the worst possible light was the one that was adopted to perform the analysis presented in Section 1.6. For this analysis, hypothetical "average" subjects were created, and the sampling distribution of SL and SA (transformed) was estimated using the "worst case" models when the value of BRTH_N = 0. The 95th percentile of each sampling distribution was used as a critical value, and the probability of exceeding this critical value was computed as the value of BRTH_N increased.

For the case of SA, the average probability (averaged over all 24 cases) when BRTH_N = 16 $\mu g/m^3$ is 12.1%. The range for the 24 cases is 11.9% to 12.3%. For the case of SL, the average probability when BRTH_N = 16 $\mu g/m^3$ is 10.9%, with a range from 10.8% to 11.0%. This provides a measure of the "worst case" effect that having elevated levels of naphthalene in the breath would have on both SA and SL. This procedure can be duplicated for any model applied to any of the performance measurements studied. One would simply need the ability to obtain predicted values for hypothetical subjects along with the standard error for prediction for various levels of the exposure variable of interest.

It has been noted by Dr. Grace Lemasters, also of the University of Cincinnati, that all of the models include variables in addition to BRTH_N that may measure exposure to JP8. These variables are BASEID, MTHBASE and MTHJOB. This is an astute observation, and one that is

certainly of concern. The solution, however, is not immediately clear. If the variables are removed from the model, then any predictive power contained within these variables beyond that supplied by the other variables in the model and, in particular, BRTH_N is lost. As a consequence, it would be expected that an increase in the standard error for prediction would be observed. As the methods described here take a "worst case" approach to the problem, however, this may be an appropriate approach to the problem. Time limitations, however, do not allow full consideration of this concern. As partial consideration, however, the analyses were re-run without these variables, and the "worst case" effect of BRTH_N was, in all cases, only slightly larger. Consider, for example, the "worst case" effect of BRTH_N for the chosen models on ln(SA) and 2*ln(SL)-6.5. For ln(SA), the upper limit of the 95% confidence interval for the coefficient was originally 0.01204. When the variables are removed, the upper limit is 0.01538. The original coefficient corresponds to an increase (in the log scale) of SA of 0.19264 while the new coefficient corresponds to an increase of 0.24608 when BRTH_N = 16 $\mu g/m^3$. Similarly for 2*ln(SL)-6.5 as the response variable, the coefficient was originally 0.01324, while the new coefficient would be 0.01598, corresponding to an increase in SL (log scale) from 0.21184 to 0.25568. These are, in reality, minor differences.

Dr. Lemasters also pointed out another method for handling levels of BRTH_N that are below the 0.5 $\mu g/m^3$ limit. It may be reasonable to assign values of 0.5 $\mu g/m^3$ for those subjects which have CATEGORY = "HI", while assigning values of 0 $\mu g/m^3$ for those subjects which have CATEGORY = "LOW" or "MOD". Again, this is a completely valid concern, and one that may be addressed in the future. Time limitations again do not allow for full consideration of this concern.

		GENDER	
BASEID	SESSION	M	F
DAV	Pre	5.33178	6.12744
	Post	5.22973	6.01094
HUR	Pre	5.35709	6.17220
	Post	5.25489	6.05574
LAN	Pre	4.09057	4.69633
	Post	4.01221	4.60689
LIT	Pre	5.05244	5.81239
	Post	4.95564	5.70188
POP	Pre	5.33380	6.12689
	Post	5.23162	6.01031
SEY	Pre	5.21319	5.98667
	Post	5.11332	5.87256

Table 1.7: 95^{th} percentiles for SA (in cm^2) for the estimated predictive distribution when BRTH_N = 0.

		GENDER	
BASEID	SESSION	M	F
DAV	Pre	52.7439	52.1613
	Post	51.5577	50.9794
HUR	Pre	52.6497	52.1731
	Post	51.4572	50.9839
LAN	Pre	46.1965	45.6569
	Post	45.1435	44.6089
LIT	Pre	52.6095	52.0673
	Post	51.4234	50.8851
POP	Pre	52.4136	51.8709
	Post	51.2269	50.6877
SEY	Pre	53.7810	53.1786
	Post	52.5663	51.9690

Table 1.8: 95^{th} percentiles for SL (in cm) for the estimated predictive distribution when BRTH_N = 0.

Chapter 2
GASH/BARS

2.1 Introduction

The next set of data presented here is the GASH/BARS data collected by the group headed by Dr. Kent Anger, Oregon Health Sciences University, Maj. Don Christensen, and Lt. Col. Roger Gibson, AFIERA. A battery of tests was given to each of the subjects participating in this portion of the study known as the GASH/BARS neurobehavioral performance tests. Table 2.1 lists the breakdown by base and dates during which the tests were conducted. This table only lists those subjects that were included in the data set. A number of subjects were removed for a number of different reasons. These reasons are not outlined here. Prior to performing his or her assigned tasks, each subject underwent the GASH/BARS tests. After performing his or her tasks, the subject then underwent the same set of tests.

It is of interest to determine the extent to which exposure to JP8 has an effect on a subject's performance on these tests. To do this, a model is built to relate two of the performance measurements (match-to-sample and tapping tests) to the various covariates using the SAS system.

Base	Subjects Tested	Dates
Davis Monthan	63	April 10-14, 2000
Hurlburt Field	41	September 18-21, 2000
Langley	58	June 12-16, 2000
Little Rock	49	August 21-25, 2000
Pope	60	July 17-21, 2000
Seymour Johnson	54	May 15-19, 2000

Table 2.1: Numbers of Subjects Examined

2.2 The Data

The match-to-sample test is a test of short-term memory. A sample stimulus is presented, followed by three choices. One of the three choices is the same as the sample. The subject is instructed to identify the stimulus that matches that which they had previously seen. The variable of interest in this report is the MS_SC score, which is the total number of correct choices the subject made on the three different match-to-sample tests administered, each with a different delay between the presentation of the stimulus and the choice.

In the tapping test, the subject is instructed to tap a button as rapidly as possible using the index finger of their preferred hand, non-preferred hand, and alternating hands. In this report, the variables of interest are TAPP1 and TAPP2, which are two repetitions of tapping with the preferred hand. TAPP1 and TAPP2 are combined to form a single variable TAPP. Thus, each subject makes up four rows in the data set: TAPP1 for SESSION = "Pre", TAPP2 for SESSION = "Pre", TAPP1 for SESSION = "Post", and TAPP2 for SESSION = "Post".

2.3 Variance-Covariance Structure

As in the postural sway measurements, each observation collected is not independent of every other observation collected. Unlike the postural sway measurements, however, the variance-covariance matrix is not as large, and choosing an appropriate variance-covariance structure is trivial.

For the match-to-sample tests, there are only two observations per subject, so the variance-covariance matrix is two by two, and it is clear that the best choice is an unstructured matrix. The number of free variance-covariance parameters is three in this case.

For the tapping tests, there are four observations per subject (TAPP1 and TAPP2, both collected during SESSION = "Pre" and SESSION = "Post"). The variance-covariance matrix is four by four, yielding a maximum total of 10 variance-covariance parameters. Again, because of the small number of parameters, the unstructured matrix is used.

2.4 Model Selection

Linear models are fit to MS_SC and TAPP (no transformations are required in this case) for the following covariates: BASEID, SESSION, BRTH_N, AGE, GENDER, MTHBASE, MTHJOB, RACE, PEXERT, MENTAL, HEIGHT, WEIGHT, BMI, SMOKER, ALCOHOL, PHYSWRK, PHYSNTWK, AFQTADMN, AFQTELCT, AFQTGEN, AFQTMECH, PFSCORE, SFSCORE, PHYSCORE, EMSCORE, MHSCORE, EFSCORE, PAINSCRE, GHPSCORE, and GHCHSCRE. Again, full models are run, and insignificant variables are removed one at a time to yield a simplified model. The variable BRTH_N is retained regardless of its significance. In both cases, the reduced model has a value of R^2 that is reduced less than 0.2% as compared to the full model. The same concerns regarding the unobservable values of BRTH_N apply, and these values are handled in the same way as in the postural sway analysis. For both models, assuming BRTH_N to be equal to ½ $\mu g/m^3$ is the "worst case" model.

The results for the final models are summarized in Table 2.2. Summary statistics for the model are again given as well as the estimated and lower end of the 95% confidence interval for the coefficient associated with BRTH_N. The lower end of the 95% confidence interval is used in this case since lower values of MS_SC and TAPP represent, in this case, a poorer performance.

2.5 Model Validity

The assumptions (normality and constant variance) are tested in the same manner as that for the postural sway analysis. The tests for normality for the MS_SC analysis reject the assumption of normality in both cases (SESSION = "Pre" and "Post"), so the p-values given in Table 2.2 should be viewed with some caution. The sample size is quite large, however, so the results given that rely on this normality assumption can be viewed as approximate. Viewing the residual versus fitted plot for both groups gives no reason to suspect the variance to be non-constant. They do, perhaps, shed light on the non-normality. It appears there is a group of 4 or 5 observations that have residuals that are smaller than would be expected from a normally distributed set of observations. That is, the distribution of the residuals appears to be slightly skewed.

	Response Variable	
Effect	MS_SC	TAPP
BRTH_N	0.2857	0.0622
SESSION		<0.0001
MTHBASE		0.0435
GENDER		0.0003
PEXERT		0.0394
PHYSWRK		0.0085
MENTAL	0.0285	
AFQTGEN	0.0015	0.0392
AFQTMECH		0.0163
Log Likelihood	-1641.7	-4397.8
Akaike	-1644.7	-4407.8
Var Params	3	10
R^2	0.97651	0.97676
BRTH_N Coefficient	-0.08690	-0.39510
(Lower 95% CL)	-0.24613	-0.80806

Table 2.2: Summary of PROC MIXED for MS_SC and TAPP. (Note: The value of BRTH_N used if it is actually below the observable limit is ½ $\mu g/m^3$. Effects with empty cells were not included in the model.)

The tests for normality for the TAPP analysis reject for the two repetitions when SESSION = "Pre", but fail-to-reject for SESSION = "Post". The residual versus fitted plot for all four groups again gives no reason to suspect a non-constant variance. As before, there appears to be a group of 3 or 4 observations that have residuals smaller than would be expected from the normal distribution. The conclusion is again the same; the distribution of the residuals is likely slightly skewed.

2.6 Effects of BRTH_N on MS_SC and TAPP

As before, hypothetical subjects are created and compared using the "worst case" models. Because the model for MS_SC only includes three variables, a larger number of hypothetical subjects can be studied. A total of 9 different hypothetical subjects were created consisting of all possible combinations of MENTAL = {3, 4, 5} and AFQTGEN = {48, 61, 72} (those being the 1st, 2nd, and 3rd quartiles, respectively, for both MENTAL and AFQTGEN). BRTH_N is again allowed to vary from 0 $\mu g/m^3$ to 16 $\mu g/m^3$, and the estimated probabilities of exceeding the computed "critical value" (estimated 95th percentile of the distribution of responses when BRTH_N = 0 $\mu g/m^3$) are computed. These probabilities for all 9 different hypothetical subjects are plotted in Figure 2.1. There is very little difference between the plotted lines. In every case, the probability is approximately 19% when BRTH_N = 16 $\mu g/m^3$, which is a 14% increase over the 5% probability when BRTH_N = 0 $\mu g/m^3$. Again, it is important at this point to keep in

mind that the tests for normality for the residuals rejected the null hypothesis, so these probabilities should be viewed with caution. The 95th percentiles used to compute the probabilities in Figure 2.1 are given in Table 2.3.

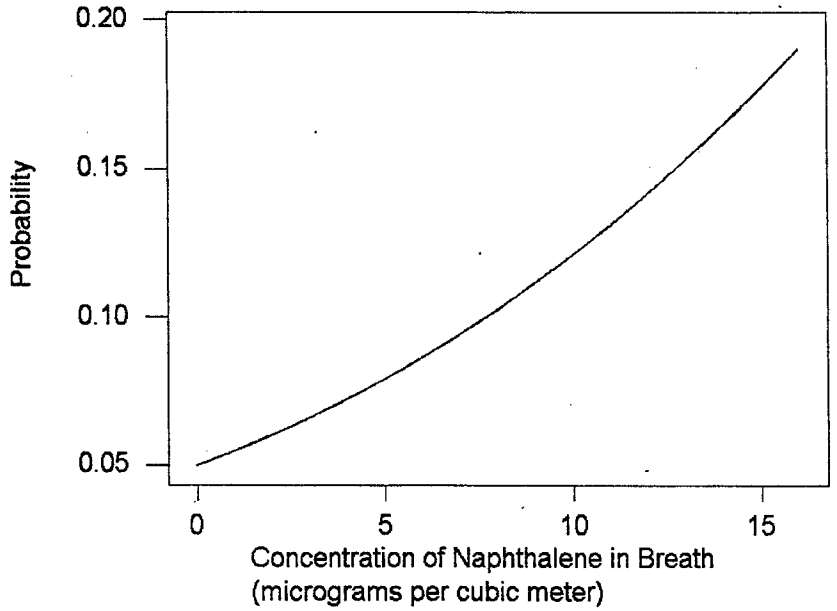

Figure 2.1 Probability of exceeding 95th percentile of MS_SC as a function of BRTH_N.

MENTAL	AFQTGEN	95th Percentile
3	48	22.8713
	61	23.5394
	72	24.0943
4	48	22.5254
	61	23.1950
	72	23.7511
5	48	22.1719
	61	22.8429
	72	23.4003

Table 2.3: 95th percentiles for MS_SC for the estimated predictive distribution when BRTH_N = 0.

For TAPP, there are seven significant variables (eight if BRTH_N with a p-value of 0.0622 is considered significant), so the number of hypothetical subjects is kept small. A male and female subject are considered, where the male subject has MTHBASE = 36, PEXERT = 8, PHYSWRK = 2, MENTAL = 3, AFQTGEN = 59, and AFQTMECH = 66. The female subject has

MTHBASE = 36, PEXERT = 6, PHYSWRK = 3, MENTAL = 3, AFQTGEN = 63, and AFQTMECH = 53. The values for PEXERT, PHYSWRK, MENTAL, AFQTGEN, and AFQTMECH are chosen to be the median scores for the respective genders. Since BRTH_N is marginally significant in this case, it is also instructive to consider the increase in the probabilities for the non-adjusted case. That is, using the actual predicted coefficient for BRTH_N in addition to the "worst case" adjusted coefficient. These are plotted in Figure 2.2. The lower set of lines is, obviously, the non-adjusted case. For each set of four lines, the two that are slightly lower are from the "Post" SESSION. For the "worst case" adjusted set, the probability increases to 19.4% for SESSION = "Post" and to 20% for SESSION = "Pre", while for the unadjusted set, the probability increases to 10.5% for SESSION = "Post" and to 10.8% for SESSION = "Pre". The 95th percentiles used to compute the probabilities in Figure 2.2 are given in Table 2.4.

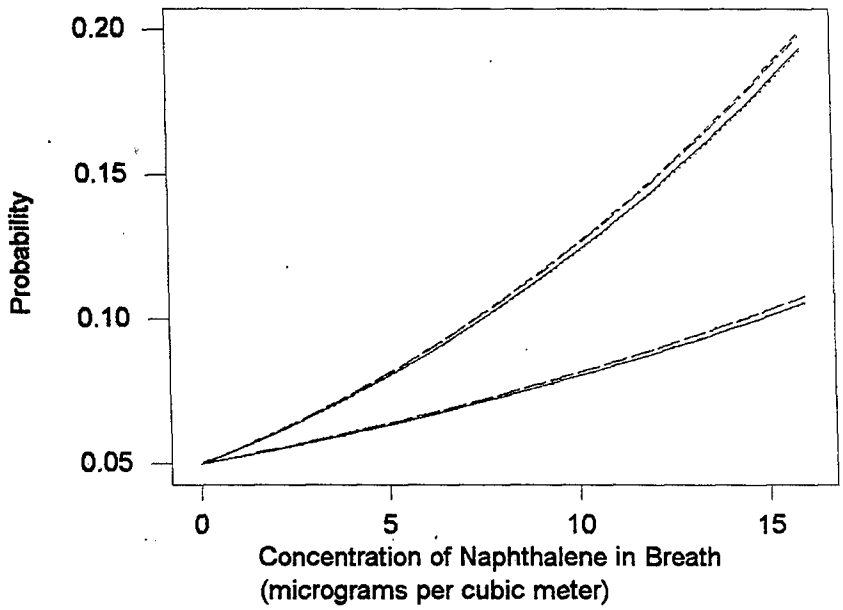

Figure 2.2 Probability of exceeding 95th percentile of TAPP as a function of BRTH_N.

	GENDER	
SESSION	M	F
Pre	77.8641	72.9507
Post	82.6696	77.7620

Table 2.4: 95th percentiles for TAPP for the estimated predictive distribution when BRTH_N = 0.

2.7 Conclusions

Mixed models were constructed to predict for the variables MS_SC and TAPP based on the covariates BASEID, SESSION, BRTH_N, AGE, GENDER, MTHBASE, MTHJOB, RACE, PEXERT, MENTAL, HEIGHT, WEIGHT, BMI, SMOKER, ALCOHOL, PHYSWRK, PHYSNTWK, AFQTADMN, AFQTELCT, AFQTGEN, AFQTMECH, PFSCORE, SFSCORE, PHYSCORE, EMSCORE, MHSCORE, EFSCORE, PAINSCRE, GHPSCORE, and GHCHSCRE. The insignificant covariates were removed one at a time to yield reduced models for MS_SC and TAPP. The models that cast the effect of BRTH_N in the worst possible light were the ones that were adopted to perform the analysis presented in Section 2.6. Again, hypothetical subjects were created, and the sampling distribution of MS_SC and TAPP were estimated when BRTH_N = 0 $\mu g/m^3$ using the "worst case" models in both cases, and in the case of TAPP, the non-adjusted model as well. The 95^{th} percentile of each sampling distribution was used as a critical value, and the probability of exceeding this critical value was computed as the value of BRTH_N increased.

For the case of MS_SC, the average probability was 19.02% when BRTH_N = 16 $\mu g/m^3$ with a range of 18.99% to 19.03%. For TAPP, the average probability was 19.6% with a range of 19.3% to 20.0%. Also for TAPP, the un-adjusted estimated probabilities had an average of 10.7% with a range of 10.5% to 10.8%. As before, this provides some measure of the "worst case" effect that having elevated levels of naphthalene in the breath would have on both MS_SC and TAPP.

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UNCERTAINTIES

There are a number of factors that contribute to the uncertainties of JP8 jet fuel risk assessments. These can be divided primarily into those factors associated with exposures and effects. The current assessment was conducted because of consideration of the health effect of exposure to JP8 jet fuel revealed several important uncertainties and questions.

Currently, little is known about the relative importance of potential exposure routes for or about uptake, absorption, tissue distribution, and elimination of JP8 following exposure of fuel worker in the occupational environment. The relation of relevant JP8 concentrations to biologically effective concentrations is also an uncertainty, yet necessary for determination of relevant risks. An assessment of developmental effects requires knowledge of appropriate, critical, life stages (e.g. embryonic, larval, juvenile, and adult) during which animals are exposed in the environment, which would require an animal model to address these uncertainties.

Due to the chemical properties of components of JP8, they are likely to bioaccumulate from environmental exposures; however, there is limited information on chronic exposures and bioaccumulation, which is likely to affect the outcomes of risk assessments. Implementation of a prospective epidemiological study of fuel workers entering the service could provide the scientific data to reduce these uncertainties.

The interpretation of the potential effects of JP8 is difficult under acute conditions when considering the uncertainty that deals with the co-occurrence of toxicants and the potential for synergistic effects. To address these uncertainties the JP8 research team that is currently in place should be funded to continue the acute risk assessment followed by designing and implementing a chronic prospective epidemiological study of JP8 jet fuel.